CHAPTER 1. DISPOSITION AND PHARMACOKINETICS

The disposition and pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds have been investigated in several species and under various exposure conditions. Several reviews on this subject focus on 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983; Birnbaum, 1985; van den Berg et al., 1994). The relative biological and toxicological potency of 2,3,7,8-TCDD and related compounds depends not only on the affinity of these compounds for the aryl hydrocarbon receptor (AhR), but on the species-, strain-, and congener-specific pharmacokinetics of these compounds (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983; Birnbaum, 1985; van den Berg et al., 1994, DeVito and Birnbaum, 1995). During the past 6 years, considerably more data have been published on this class of compounds, which includes 2,3,7,8-substituted CDDs, BDDs, CDFs, BDFs, and the coplanar PCBs and PBBs. This chapter reviews the disposition and pharmacokinetics of these agents and identifies congener- and species-specific factors that may have an impact on the dose-related biological responses of these compounds.

1.1. ABSORPTION/BIOAVAILABILITY FOLLOWING EXPOSURE

Gastrointestinal, dermal, and transpulmonary absorptions represent potential routes for human exposure to this class of persistent environmental contaminants. Parenteral absorption is a route of exposure that has been used to generate disposition and pharmacokinetic data on these compounds.

1.1.1. Oral

1.1.1.1. *Gastrointestinal Absorption in Animals*

Much of human exposure to 2,3,7,8-TCDD and related compounds is thought to be through the diet. Experimentally, these compounds are commonly administered in the diet or by gavage in an oil vehicle. Gastrointestinal absorption is usually estimated as the difference between the administered dose (100%) and the percent of the dose that was not absorbed. The unabsorbed fraction is estimated as the recovery of parent compound in feces within 24 to 48 hours of a single oral exposure by gavage. Table 1-1 summarizes gastrointestinal absorption data on 2,3,7,8-TCDD and related compounds.

In Sprague-Dawley rats given a single oral dose of 1.0 μg [^{14}C]-2,3,7,8-TCDD/kg bw in acetone:corn oil (1:25, v/v), the fraction absorbed ranged from 66% to 93%, with a mean of ~84% (Rose et al., 1976). With repeated oral dosing of rats at 0.1 or 1.0 μg/kg/day (5 days/week for 7 weeks), gastrointestinal absorption of 2,3,7,8-TCDD was observed to be approximately that observed for a single oral exposure (Rose et al., 1976). Oral exposure of Sprague-Dawley rats to

a larger dose of 2,3,7,8-TCDD in acetone:corn oil (50 μg/kg) resulted in an average absorption of 70% of the administered dose (Piper et al., 1973). More recently, Diliberto et al. (1996a) reported 88% absorption of 2,3,7,8-TCDD in male Fischer 344 rats following oral exposure in Emulphor/95% ethanol/water (1:1:3). (Emulphor EL-620 is a polyoxyethylated vegetable oil preparation [GAF Corp., New York, NY]).

One study in the guinea pig reported that ~50% of a single oral dose of 2,3,7,8-TCDD in acetone:corn oil was absorbed (Nolan et al., 1979). The gastrointestinal absorption of 2,3,7,8-TCDD was also examined in the hamster, the species most resistant to the acute toxicity of this compound (Olson et al., 1980). Hamsters were given a single, sublethal, oral dose of [1,6-^{3}H]-2,3,7,8-TCDD in olive oil (650 μg/kg), and an average of 75% of the dose was absorbed. When 2,3,7,8-TCDD was administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 μg/kg/day) for 42 days, 50% to 60% of the consumed dose was absorbed (Fries and Marrow, 1975). These findings indicate that oral exposure to 2,3,7,8-TCDD in the diet or in an oil vehicle results in the absorption of >50% of the administered dose.

The intestinal absorption of [^{3}H]-2,3,7,8-TCDD has also been investigated in thoracic duct-cannulated rats (Lakshmanan et al., 1986). The investigators concluded that 2,3,7,8-TCDD was absorbed into chylomicrons and transported through the lymphatic system before entering the systemic circulation.

The absorption of 2,3,7,8-TBDD in male Fischer 344 rats was studied after oral exposure by gavage at 5 μg/kg in Emulphor:ethanol:water (1:1:3) (Diliberto et al., 1990). The percent of the dose absorbed for this study was defined as 100% (% total oral dose in feces on days 1 and 2 minus % total intravenous dose in feces on days 1 and 2) using the intravenous pharmacokinetic data of Kedderis et al. (1991a).

The relative absorbed dose or bioavailability of 2,3,7,8-TBDD after oral exposure was estimated at 78%, 82%, 60%, and 47% at dose levels of 0.001, 0.01, 0.1, and 0.5 μmol/kg, respectively. These results suggest nonlinear absorption at the higher doses, with maximal oral absorption at an exposure of ≤0.01 μmol/kg (5 μg/kg).

The absorption of 2,3,7,8-TCDF has been investigated after oral exposure by gavage. Approximately 90% of the administered dose (0.1 and 1.0 μmol/kg) of 2,3,7,8-TCDF in Emulphor:ethanol (1:1) was absorbed in male Fischer 344 rats (Birnbaum et al., 1980). Similarly, >90% of the administered dose (0.2 μmol/kg, 6 μg/kg, and 1-15 μg/kg) of 2,3,7,8-TCDF in Emulphor:ethanol:water (1:1:8) was absorbed in male Hartley guinea pigs (Decad et al., 1981a; Ioannou et al., 1983). Thus, 2,3,7,8-TCDF appears to be almost completely absorbed from the gastrointestinal tract. This absorption may be related to the greater relative solubility of 2,3,7,8-TCDF compared with that of 2,3,7,8-TCDD or 2,3,7,8-TBDD.

The oral bioavailability of 2,3,4,7,8-PeCDF and 3,3′,4,4′-TCB in corn oil was similar to that of 2,3,7,8-TCDD (Brewster and Birnbaum, 1987; Wehler et al., 1989; Clarke et al., 1984). Furthermore, 2,3,4,7,8-PeCDF absorption was independent of the dose (0.1, 0.5, or 1.0 μmol/kg). Incomplete and variable absorption of 1,2,3,7,8-PeCDD was reported in rats, with 19% to 71% of the dose absorbed within the first 2 days after oral exposure (Wacker et al., 1986).

Early studies on the pharmacokinetic behavior of OCDD by Williams et al. (1972) and Norback et al. (1975) demonstrated that OCDD was poorly absorbed after oral exposure. More recently, Birnbaum and Couture (1988) also found that the gastrointestinal absorption of OCDD in rats was very limited, ranging from 2% to 15% of the administered dose. Lower doses (50 μg/kg) in an o-dichlorobenzene:corn oil (1:1) vehicle were found to give the best oral bioavailability for this extremely insoluble compound.

Recently Mes et al. (1995) have reported PCBs in nondosed infants of dosed rhesus monkeys. Transfer to the infants was from both intrauterine and lactational exposure. Also, the infants showed a larger percentage of heptachorobiphenyls than did the dosed dams. Busbee and Zipring (1994) reported the direct absorption across the gastric mucosa of Aroclor 1232 (DCB) in an ovine. The absorption was rapid and the circulating DCB was not found to be associated with plasma lipid fractions. The researchers report no occurrence in the thoracic duct lymph prior to appearance in the circulating plasma. Also, the DCB did bind with plasma lipids in vitro. These data suggest that this DCB is transported similar to water soluble compounds.

1.1.1.2. *Gastrointestinal Absorption in Humans*

The above animal data indicate that gastrointestinal absorption of 2,3,7,8-TCDD and related compounds is variable, incomplete, and congener- and vehicle-specific. More soluble congeners, such as 2,3,7,8-TCDF, are almost completely absorbed, whereas the extremely insoluble OCDD is poorly absorbed. In some cases, absorption has been found to be dose dependent, with increased absorption occurring at lower doses (2,3,7,8-TBDD, OCDD). The limited database in experimental animals also suggests that there are no major interspecies differences in the gastrointestinal absorption of these compounds.

Poiger and Schlatter (1986) investigated the absorption of 2,3,7,8-TCDD in a 42-year-old man after ingestion of 105 ng [^{3}H]-2,3,7,8-TCDD (1.14 ng/kg bw) in 6 mL corn oil and found that >87% of the oral dose was absorbed from the gastrointestinal tract. Following absorption, the half-life for elimination was estimated to be 2,120 days.

Schlummer et al. (1998) used a mass balance approach to assess the gastrointestinal absorption of CDDs, CDFs, PCBs, and hexachlorobenzene (HCB) from food in seven individuals, 24 to 81 years of age, with different contaminant body burdens. The difference between the

ingested (food concentration) and excreted (fecal concentration) amounts of each compound was defined as net absorption. Three types of net absorption were observed in this study: (1) nearly complete net absorption (e.g. 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF); (2) incomplete net absorption (e.g., 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in the younger subjects); and (3) net excretion (excretion to a greater extent than ingestion, e.g., 1,2,3,6,7,8-HxCDD and OCDD). In terms of TEQs, the maximum net absorption of CDDs and CDFs was 63% in one individual, while a net excretion of TEQs was found for the three oldest subjects, which also have the highest serum concentration of these congeners. When PCB 126 (3,3'4,4',5), with a TEF of 0.1, is included in the TEF calculation, the TEQ balance was dominated by this congener, resulting in a maximum net TEQ absorption of 80% and a net TEQ absorption in all but the oldest subject. Table 1-1 illustrates that compounds showing nearly complete net absorption had very low or nondetectable levels in the serum lipids, and for other congeners, there was a trend for decreasing net absorption/increasing net excretion with increasing congener concentration in serum lipids. Together, the data support the passive diffusion model for gastrointestinal absorption, where the concentration of the contaminant in the blood is the major factor determining absorption. However, the relatively high absorption levels of many congeners could not be explained on the basis of diffusive gradients estimated from the difference between the lipid-based food and serum concentrations, as the lipid-based food levels were always lower, favoring net excretion. The authors propose a fat-flush theory, which hypothesizes that the fat compartment of the absorbing tissue (gut wall) expands because of the uptake of dietary fat, resulting in a decrease in the lipid-based concentration of the gut wall below that of the food, thus facilitating absorption. Therefore, as food passes through the duodenum and the jejunum, CDDs, CDFs and PCBs experience a diffusion gradient and net absorption as a result of the fat-flush. As the gut contents reach the colon, dietary fat has been absorbed, causing a reduction in the concentration gradient favoring absorption of CDDs, CDFs, and PCBs. Thus, the fat-flush theory supports the hypothesis that absorption and excretion of CDDs, CDFs, and PCBs are distinct processes occurring at different locations in the digestive tract.

Duarte-Davidson and Jones (1994) report an average intake of a sum of several PCB congeners in the contemporary United Kingdom population of 0.53 micrograms/person/day. They estimate 97% of exposure can be accounted for by food consumption. This indicates that PCBs can likewise be absorbed through the gastrointestinal system.

Because CDDs, CDFs, and PCBs are present in human milk, McLachlan (1993) investigated the net absorption of these compounds in a nursing infant. The contaminant input, through the ingestion of mother's milk, and the contaminant output in the feces were measured to estimate the digestive tract absorption of these compounds. For almost all congeners, more than 90% of the ingested compound was absorbed, indicating that the common assumption of 100%

absorption of CDDs, CFFs, and PCBs in nursing infants is reasonable. Dahl et al. (1995) provide further evidence of this, as they report > 95% absorption in postpartum infants (1, 2, 3 months) in Sweden. Abraham et al. (1996) assessed the oral intake and fecal excretion of CDDs and CDFs in one formula-fed and two breast-fed infants at 1 and 5 months of age. The breast-fed infants had significantly more exposure to CDDs and CDFs, with >90% of the 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, and 1,2,3,6,7,8-HxCDD (>93% of TEQs) being absorbed from mother's milk. Less complete bioavailability of higher CDDs was observed, with 62% to 88% of 1,2,3,4,6,7,8-HepCDD and 16% to 75% of OCDD absorbed from mother's milk (Abraham et al., 1996). Furst et al. (1994), Hong et al. (1994), Schecter et al. (1994a,b), Georgii et al. (1994), and others provide further evidence of the presence of dioxins, PCPs, and PCBs in human milk. This important route for excretion and exposure is discussed later in the chapter.

1.1.1.3. *Bioavailability Following Oral Exposure*

Oral exposure of humans to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in food, soil, dust, water, or other mixtures that would be expected to alter absorption.

The influence of dose and vehicle or adsorbent on gastrointestinal absorption has been investigated in rats by Poiger and Schlatter (1980), using hepatic concentrations 24 hours after dosing as an indicator of the amount absorbed (Table 1-2). Administration of 2,3,7,8-TCDD in an aqueous suspension of soil resulted in a decrease in the hepatic levels of 2,3,7,8-TCDD as compared with hepatic levels resulting from administration of 2,3,7,8-TCDD in 50% ethanol. Likewise, Diliberto et al. (1996a) report 24.4% (+ 1.4) in the liver after dosing male Fischer 344 rats with 2,3,7,8-TCDD in a solution of 1:1:3 ratio of Emulphor/95% ethanol/distilled water. The extent of the decrease was directly proportional to the length of time the 2,3,7,8-TCDD had been in contact with the soil. When 2,3,7,8-TCDD was mixed in an aqueous suspension of activated carbon, absorption was almost totally eliminated (<0.07% of the dose in hepatic tissues).

Philippi et al. (1981) and Huetter and Philippi (1982) have shown that radiolabeled 2,3,7,8-TCDD becomes progressively more resistant with time to extraction from soil. Similarly, the feeding of fly ash, which contains CDDs, to rats in the diet for 19 days resulted in considerably lower hepatic levels of CDDs than did the feeding of an extract of the fly ash at comparable dietary concentrations of CDDs (van den Berg et al., 1987a). The CDDs were tentatively identified as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD, and the difference in hepatic levels noted between fly ash-treated and extract-treated rats was greater for the more highly chlorinated isomers than for 2,3,7,8-TCDD. These results indicate the importance of the formulation or vehicle containing the toxin(s) on the relative bioavailability of 2,3,7,8-TCDD, PeCDD, and HxCDDs after oral exposure.

Because 2,3,7,8-TCDD in the environment is likely to be absorbed to soil, McConnell et al. (1984) and Lucier et al. (1986) compared the oral bioavailability of 2,3,7,8-TCDD from environmentally contaminated soil with that from 2,3,7,8-TCDD administered in corn oil in guinea pigs and rats, respectively. As indicated by biological effects and the amount of 2,3,7,8-TCDD in the liver, the intestinal absorption from soil from Times Beach and Minker Stout, Missouri, was ~50% less than that from corn oil. Shu et al. (1988a) reported an oral bioavailability of ~43% in rats dosed with three environmentally contaminated soil samples from Times Beach, Missouri. This figure did not change significantly over a 500-fold dose range of 2-1,450 ng 2,3,7,8-TCDD/kg bw for soil contaminated with ~2, 30, or 600 ppb of 2,3,7,8-TCDD. In studies of other soil types, Umbreit et al. (1986a,b) estimated an oral bioavailability in the rat of 0.5% for soil at a New Jersey manufacturing site and 21% for a Newark salvage yard. These results indicate that bioavailability of 2,3,7,8-TCDD from soil varies between sites and that 2,3,7,8-TCDD content alone may not be indicative of potential human hazard from contaminated environmental materials. Although these data indicate that substantial absorption may occur from contaminated soil, soil type and duration of contact, as suggested from the data that demonstrated decreased extraction efficiency with increasing contact time between soil and 2,3,7,8-TCDD (Philippi et al., 1981; Huetter and Philippi, 1982), may substantially affect the absorption of 2,3,7,8-TCDD from soils obtained from different contaminated sites.

1.1.2. Dermal Absorption

Brewster et al. (1989) examined the dermal absorption of 2,3,7,8-TCDD and three CDFs in male Fischer 344 rats (10 weeks old; 200-250 g). The fur was clipped from the intrascapular region of the back of each animal. A single compound in 60 μL of acetone was then applied over a 1.8 cm^2 area of skin, which then was covered with a perforated stainless steel cap. Table 1-3 summarizes data on the absorption of each compound at 3 days after a single dermal exposure. At an exposure of 0.1 μmol/kg, the absorption of 2,3,7,8-TCDF (49% of administered dose) was greater than that of 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF, and 2,3,7,8-TCDD. For each compound, the relative absorption (percentage of administered dose) decreased with increasing dose, while the absolute absorption (μg/kg) increased nonlinearly with dose. Results also suggest that the majority of the compound remaining at the skin exposure site was associated with the stratum corneum and did not penetrate through to the dermis. In a subsequent study, Banks and Birnbaum (1991a) examined the rate of absorption of 2,3,7,8-TCDD over 120 hours after the dermal application of 200 pmol (1 nmol/kg) to male Fischer 344 rats. The absorption kinetics appeared to be first order, with an absorption rate constant of 0.005 $hour^{-1}$. With a similar exposure protocol, the dermal absorption of 2,3,7,8-TCDF was found to follow a first-order process, with a rate constant of 0.009 $hour^{-1}$ (Banks and Birnbaum, 1991b). Together, these

results on dermal absorption indicate that at lower doses (≤0.1 µmol/kg), a greater percentage of this administered dose of 2,3,7,8-TCDD and three CDFs was absorbed. Nonetheless, the rate of absorption of 2,3,7,8-TCDD is still very slow (rate constant of 0.005 $hour^{-1}$), even following a low-dose dermal application of 200 pmol (1 nmol/kg). Results from Table 1-3 also suggest that the dermal absorption of 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and 1,2,3,7,8-PeCDF occurs at a very slow rate. Using a similar exposure protocol, the dermal absorption of 2,3,7,8-TBDD was only 30% to 40% of that observed for 2,3,7,8-TCDD (Diliberto et al., 1993a; 1996a).

The dermal absorption of several polyhalogenated aromatic hydrocarbons in male F344 rats was also compared with estimates of their respective octanol-water partition coefficients (Jackson et al., 1993). Inverse correlations were found between octanol-water partition coefficient estimates and the single-dose (~1.0 nmol/kg and ~0.1 µmol/kg) dermal absorption for most of the compounds studied. The differential dermal absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD may result, in part, from the diminished ability of the more lipophilic 2,3,7,8-TBDD to partition out of the stratum corneum and into the underlying epidermal and dermal layers.

Rahman et al. (1992) and Gallo et al. (1992) compared the in vitro permeation of 2,3,7,8-TCDD through hairless mouse and human skin. In both species, the amount of 2,3,7,8-TCDD permeated increased with the dose, but the percentage of the dose permeated decreased with increasing dose. The permeability coefficient of 2,3,7,8-TCDD in human skin was about one order of magnitude lower than that in mouse skin. The hairless mouse skin does not appear to be a suitable model for the permeation of 2,3,7,8-TCDD through human skin because the viable tissues were major barriers to 2,3,7,8-TCDD permeation in hairless mouse skin, while the stratum corneum layer provided the greater resistance in human skin. A significant increase in 2,3,7,8-TCDD permeation through human skin was observed when the skin was damaged by tape-stripping. Gallo et al. (1992) suggested that washing and tape-stripping of the exposed area might remove most of the 2,3,7,8-TCDD and reduce the potential for systemic exposure and toxicity because most of the 2,3,7,8-TCDD remained within the horny layer of human skin even at 24 hours following exposure.

Weber et al. (1991) also investigated the penetration of 2,3,7,8-TCDD into human cadaver skin at concentrations of 65-6.5 ng/cm^2. This study found that the stratum corneum acted as a protective barrier, as its removal increased the amount of 2,3,7,8-TCDD absorbed into layers of the skin. With intact skin and acetone as the vehicle, the rate of penetration of 2,3,7,8-TCDD into the dermis ranged from 6 to 170 $pg/hour/cm^2$, while penetration into the dermis and epidermis ranged from 100 to 800 $pg/hour/cm^2$. With mineral oil as the vehicle, there was about a five- to tenfold reduction in the rate of penetration of 2,3,7,8-TCDD into the intact skin.

Wester et al. (1993a) studied the dermal permeation potential of two PCBs, Aroclor 1242 and Aroclor 1254, from soil. Soil is the most common medium of contact for humans; hence any

permeation potential is of great interest for risk assessment purposes. This study consisted of experiments conducted in rhesus monkeys (in vivo), in vitro human skin, and powdered human corneum. The monkeys were exposed topically for a 5-week period. Percutaneous absorption was determined by urinary and fecal [^{14}C]-PCB excretion. The percutaneous absorption in the monkey was 13.8% (±2.7%) for Aroclor 1242 and 14.1% (±1.0%) for Aroclor 1254. The authors report that these absorption rates for soil are similar to those from vehicles such mineral oil, trichlorobenzene, and acetone. These rates are somewhat higher than those for other compounds such as the pesticide butachlor, which was about 5.0% when applied to human skin (Ademola et al., 1993). On the other hand, Wester et al. (1993b) report a much higher absorption for pentachlorophenol (PCP) (24.4% from soil and 29.2% from acetone) in the rhesus monkey. The authors report that PCP is one of the more extensively absorbed compounds they have studied. Thus, at least for these two PCBs (Aroclor 1242 and Aroclor 1254), the absorption in the rhesus monkey appears to be intermediate between that of other compounds tested. In summary, these studies indicate that these two PCBs show significant partitioning into the stratum corneum from soil, a common environmental matrix.

1.1.2.1. *Bioavailability Following Dermal Exposure*

Dermal exposure of humans to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in soil, oils, or other mixtures, which would be expected to alter absorption. Poiger and Schlatter (1980) presented evidence that the presence of soil or lipophilic agents dramatically reduces dermal absorption of 2,3,7,8-TCDD compared with absorption of pure compound dissolved in solvents. In a control experiment, 26 ng of 2,3,7,8-TCDD in 50 µL methanol was administered to the skin of rats; 24 hours later the liver contained 14.8±2.6% of the dose. By comparing this value to the hepatic levels obtained after oral administration in 50% ethanol (in the same study), the amount absorbed from a dermal application can be estimated at ~40% of the amount absorbed from an equivalent oral dose. This comparison assumes that hepatic levels are valid estimates of the amount absorbed from both oral and dermal routes and that absorption from methanol is equivalent to absorption from 50% ethanol. The dose-dependent distribution of 2,3,7,8-TCDD in the liver is another factor that may limit quantitative conclusions regarding bioavailability that are based solely on hepatic levels following exposure to 2,3,7,8-TCDD. As compared with dermal application in methanol, dermal application to rats of 2,3,7,8-TCDD in vaseline or polyethylene glycol reduced the percentage of the dose in hepatic tissue to 1.4% and 9.3%, respectively, but had no observable effect on the dose of 2,3,7,8-TCDD required to induce skin lesions (~1 µg/ear) in the rabbit ear assay. Application of 2,3,7,8-TCDD in a soil/water paste decreased hepatic 2,3,7,8-TCDD to ~2% of the administered dose and increased the amount required to produce skin lesions to 2-3 µg in rats

and rabbits, respectively. Application in an activated carbon/water paste essentially eliminated absorption, as measured by percent of dose in the liver, and increased the amount of 2,3,7,8-TCDD required to produce skin lesions to ~160 µg. These results suggest that dermal absorption of 2,3,7,8-TCDD depends on the formulation (vehicle or adsorbent) containing the toxin.

Shu et al. (1988b) investigated the dermal absorption of soil-bound 2,3,7,8-TCDD in rats. Relative dermal bioavailability was estimated by comparing the level of 2,3,7,8-TCDD in the liver of rats given soil-bound 2,3,7,8-TCDD dermally to that of rats given oral doses of 2,3,7,8-TCDD dissolved in corn oil. The level of 2,3,7,8-TCDD in livers of rats dosed orally with 2,3,7,8-TCDD in corn oil, following correction for unabsorbed 2,3,7,8-TCDD, is assumed to represent 100% bioavailability. The dermal penetration of 2,3,7,8-TCDD after 4 hours of contact with skin was ~60% of that after 24 hours of contact. After 24 hours of contact with the skin, the degree of dermal uptake from contaminated soil was ~1% of the administered dose. The authors observed that the degree of uptake does not appear to be influenced significantly by the concentration of 2,3,7,8-TCDD in soil, by the presence of crankcase oil as a co-contaminant, or by environmentally versus laboratory-contaminated soil.

A major limitation of these studies is the uncertainty regarding the extrapolation of dermal absorption data on these compounds from the rat to the human. The in vitro dermal uptake of 2,3,7,8-TCDD has been investigated in hairless mouse and human skin (Gallo et al., 1992; Rahman et al., 1992). In vitro dermal uptake of 2,3,7,8-TCDD from laboratory-contaminated soil indicated that aging of soils (up to 4 weeks) and the presence of additives (2,4,5-trichlorophenol and motor oil) in the soil did not have any significant effect on dermal uptake (Gallo et al., 1992). Because most of the 2,3,7,8-TCDD remained in the stratum corneum layer of human skin, the permeation of 2,3,7,8-TCDD was significantly lower in human than in hairless mouse skin. There are no published quantitative in vivo data on the dermal absorption of 2,3,7,8-TCDD and related compounds in the human, and data on the rhesus monkey are very limited. Brewster et al. (1988) found that 1,2,3,7,8-PeCDF was poorly absorbed in the monkey after dermal application, with <1% of the administered dose being absorbed in 6 hours. This provides further evidence for the very slow rate of dermal absorption of 2,3,7,8-TCDD and related compounds.

1.1.3. Transpulmonary Absorption

The use of incineration as a means of solid and hazardous waste management results in the emission of contaminated particles that may contain TCDD and related compounds into the environment. Thus, significant exposure to TCDD and related compounds may result from inhalation of contaminated fly ash, dust, and soil. In an attempt to address the bioavailability and potential health implications of inhaling contaminated particles, Nessel et al. (1990) examined the potential for transpulmonary absorption of TCDD in female Sprague-Dawley rats after

intratracheal instillation of the compound in a corn oil vehicle or as a laboratory-prepared contaminant of gallium oxide particles. Several biomarkers of systemic absorption were measured, including the dose-dependent effects of TCDD on hepatic microsomal cytochrome P-450 content, AHH activity, and liver histopathology. Significant dose-related effects were observed at an exposure of $\geq$0.55 µg TCDD/kg. The authors found that induction was slightly higher when animals received TCDD in corn oil than when animals received TCDD-contaminated particles, and was comparable to induction after oral exposure. The results from Nessel et al. (1990) indicate that systemic effects occur after pulmonary exposure to TCDD, suggesting that transpulmonary absorption of TCDD does occur.

The pulmonary bioavailability of 2,3,7,8-TCDD was also examined in female Sprague-Dawley rats following intratracheal instillation of PCDD-contaminated soil from a former 2,4,5-trichlorophenoxy-acetic acid manufacturing site (Nessel et al., 1992). A size-dependent enrichment of PCDDs and PCDFs was observed, with the smaller particles being more highly contaminated. 2,3,7,8-TCDD was enriched up to 33-fold in small respirable particles as compared with unfractionated soil. Pulmonary bioavailability of 2,3,7,8-TCDD was assessed by hepatic enzyme induction (AHH activity) and 2,3,7,8-TCDD concentration. The data indicate that the relative pulmonary bioavailability of 2,3,7,8-TCDD on respirable soil particles is 100% as compared with laboratory-recontaminated gallium oxide.

The transpulmonary absorption of 2,3,7,8-TCDD was assessed in male Fischer 344 rats following intratracheal instillation of a 1 nmol/kg dose in Emulphor:ethanol:water (1:1:3) (Diliberto et al., 1996a). Transpulmonary absorption was 95%, suggesting that there was almost complete absorption of 2,3,7,8-TCDD by inhalation under these conditions. Similar results were also observed for the transpulmonary absorption of 2,3,7,8-TBDD under similar exposure conditions (Diliberto et al., 1993a,b). Recent studies (Diliberto et al., 1996a) further show the importance of transpulmonary absorption for 2,3,7,8-TCDD. Tissue distributions were measured 3 days after administration via different routes. Comparisons show that the percentage of dose distributed to the liver after intratracheal (itr) injection is similar to that after intravenous administration (iv) (33% for itr, 37% for iv). Also, both the iv and itr routes show a preference for greater sequestration in the liver over fat when compared to the oral (po) route. These results suggest that the transpulmonary absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD was similar to that observed following oral exposure.

1.1.4. Parenteral Absorption

In an effort to obtain more reproducible and complete absorption of 2,3,7,8-TCDD and related compounds for pharmacokinetic studies, Abraham et al. (1989a,b) used various vehicles to investigate the absorption of 2,3,7,8-TCDD after parenteral application in rats. These

investigators observed optimal results with the subcutaneous injection of 2,3,7,8-TCDD with a mixture of toluene:DMSO (1:2) as the vehicle. At 3 and 5 days after treatment, the percentages of administered dose remaining at the injection site under the skin of the back were ~10% and 2%, respectively. The vehicle did not cause adverse effects at an applied volume of 0.2 mL/kg bw. The absorption of a defined mixture of CDDs and CDFs in the rat was also examined after subcutaneous injection with toluene:DMSO (1:2) as a vehicle. Of the 97 congeners analyzed, 70 were ≥95% absorbed 7 days after exposure; 21 were 90%-95% absorbed; and 1,2,3,9-TCDD, 1,2,3,6,7,9-/1,2,3,6,8,9-HxCDD, 1,2,3,4,6,7,9-HpCDD, OCDD, 1,2,4,6,8,9-HxCDF and 1,2,3,7,8,9-HxCDF were 84%-89% absorbed. Greater than 90% absorption of CDDs and CDFs was also observed under these conditions in the marmoset monkey, with the exception of 1,2,3,4,7,8,9-HpCDF, OCDF, and OCDD, which had ~50%-80% of the administered dose absorbed (Neubert et al., 1990; Abraham et al., 1989a). Although the absorption of CDDs and CDFs after subcutaneous administration in toluene:DMSO (1:2) is somewhat slow in rats and monkeys, absorption of most congeners was >90% within 7 days. Even for highly chlorinated insoluble congeners, such as OCDD and OCDF, subcutaneous absorption was >84% in the rat and >50% in the monkey.

Less complete and slower absorption of CDDs and CDFs was observed after subcutaneous injection of these compounds using an oil-containing vehicle (Brunner et al., 1989; Abraham et al., 1989a). Using a corn oil:acetone vehicle (24:1, v/v), Lakshmanan et al. (1986) observed that only 7% of the administered dose of 2,3,7,8-TCDD was absorbed 24 hours after subcutaneous injection and that only 35% was absorbed after intraperitoneal injection. Brunner et al. (1989) also reported that intraperitoneal administration of CDDs and CDFs revealed a delayed absorption from the abdominal cavity that varied for the different congeners. Therefore, concentrations measured in abdominal adipose tissue after intraperitoneal administration may not represent average values of adipose tissue in the whole body, particularly at early time points following exposure. This may also be true following oral exposure because of different perfusion rates of different fat depots (McKinley et al., 1993).

1.2. DISTRIBUTION

1.2.1. Distribution in Blood and Lymph

Once a compound is absorbed, its distribution is regulated initially by its binding to components in blood and its ability to diffuse through blood vessels and tissue membranes. Lakshmanan et al. (1986) investigated the absorption and distribution of 2,3,7,8-TCDD in thoracic duct-cannulated rats. The results suggest that after gastrointestinal absorption, 2,3,7,8-TCDD is absorbed primarily by the lymphatic route and is transported predominantly by chylomicrons. Ninety percent of the 2,3,7,8-TCDD in lymph was associated with the chylomicron

fraction. The plasma disappearance of 2,3,7,8-TCDD-labeled chylomicrons followed first-order decay kinetics, with 67% of the compound leaving the blood compartment very rapidly ($t_{½}$=0.81 minutes), whereas the remainder of the 2,3,7,8-TCDD had a $t_{½}$ of 30 minutes. 2,3,7,8-TCDD was then found to distribute primarily to the adipose tissue and the liver.

In vitro studies have investigated the distribution of 2,3,7,8-TCDD in human whole blood. Henderson and Patterson (1988) found ~80% of the compound associated with the lipoprotein fraction, 15% associated with protein (primarily human serum albumin), and 5% associated with cellular components. A subsequent in vivo investigation reported a similar distribution of 2,3,7,8-TCDD in the various fractions of human whole blood (Patterson et al., 1989). Theoretical and limited experimental data also suggest that 2,3,7,8-TCDD and related compounds may be associated with plasma prealbumin (McKinney et al., 1985; Pedersen et al., 1986). The distribution of [^{3}H]-2,3,7,8-TCDD among lipoprotein fractions from three fasting, normolipemic donors indicated a greater percentage associated with LDL (55.3% ± 9.03% SD) than with VLDL (17.4% ± 9.07% SD) or HDL (27.3% ± 10.08% SD). The distribution of 2,3,7,8-TCDD among the lipoprotein fractions was similar to that reported earlier by Marinovich et al. (1983). When the binding of 2,3,7,8-TCDD was calculated per mole of lipoprotein, it was suggested that the maximal binding capacity was exerted by VLDL, followed by LDL and HDL (Marinovich et al., 1983). The results also suggest that variations in the amounts of each lipoprotein class may alter the distribution of 2,3,7,8-TCDD among lipoproteins in a given subject. Significant species differences also exist; in the case of the rat, which has markedly lower plasma lipids compared to humans, 2,3,7,8-TCDD was distributed almost equally among the lipoprotein fractions (Marinovich et al., 1983).

Congener-specific differences have been observed for the in vivo binding of the 2,3,7,8-substituted PCDDs and PCDFs to different serum fractions in human blood (Patterson et al., 1989). Binding to the lipoproteins gradually decreased with increasing chlorine content, with about 75% of 2,3,7,8-TCDD bound to lipoproteins while approximately 45% of OCDD was bound to this fraction. In contrast, binding to other serum proteins increased with chlorine content, from approximately 20% for 2,3,7,8-TCDD to 50% for OCDD. The results indicate that the higher chlorinated PCDDs and PCDFs do not partition according to the lipid content of these blood fractions. However, Busbee and Zipring (1994) report that at least one lower chlorinated PCB, dichlorobiphenyl, absorbs and distributes with the nonlipid plasma fractions, rather than the lipid fractions as might be expected.

In addition, there is indirect evidence that suggests that the binding of 2,3,7,8-TCDD to lipoproteins may alter the pharmacokinetics and toxic potency of the compound. Marinovich et al. (1983) found that experimentally induced hyperlipidemia in rats delayed the development of overt toxicity (lethality). However, the disposition of 2,3,7,8-TCDD was not investigated under

these conditions. These investigators suggest that the release of lipoprotein-bound 2,3,7,8-TCDD is related to the metabolic turnover of lipoproteins. In hyperlipidemic rats, the turnover of VLDL and LDL is delayed significantly compared to that in normolipidemic animals, and this may contribute to the plasma lipoprotein binding modifying the toxicity of 2,3,7,8-TCDD in hyperlipidemic rats.

The time- and temperature-dependent cellular uptake of lipoprotein-associated 2,3,7,8-TCDD by cultured human fibroblasts was greatest from LDL, intermediate from HDL, and least from serum (Shireman and Wei, 1986). Decreased cellular uptake of LDL and 2,3,7,8-TCDD was observed in mutant fibroblasts, which lack the normal cell membrane receptor for LDL. This provides some evidence that specific binding of LDL and the LDL receptor pathway may account for some of the rapid early uptake of 2,3,7,8-TCDD with LDL entry. The results suggest that the entry of 2,3,7,8-TCDD into cells may not be solely by simple diffusion. However, nonspecific binding of the LDL and transfer of 2,3,7,8-TCDD from LDL to the cell membranes are probably also important, as significant time- and temperature-dependent uptake of 2,3,7,8-TCDD and LDL occurred in the mutant fibroblasts.

Thus, upon absorption, 2,3,7,8-TCDD and probably related compounds are bound to chylomicrons, lipoproteins, and other serum proteins that assist in distributing these uncharged, lipophilic compounds throughout the vascular system. These compounds then partition from blood components into cellular membranes and tissues, probably largely by passive diffusion. In addition, cellular uptake may be facilitated partly through the cell membrane LDL receptor, the hepatic receptor for albumin (Weisiger et al., 1981), and other systems.

1.2.2. Tissue Distribution

Once absorbed into blood, 2,3,7,8-TCDD and related compounds readily distribute to all organs. Tissue distribution within the first hour after exposure parallels blood levels and reflects physiological parameters such as blood flow to a given tissue and relative tissue size. For example, high initial concentrations of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF, and 3,3′,4,4′-TCB were observed in highly perfused tissue such as the adrenal glands during the 24-hour period after a single exposure (Birnbaum et al., 1980; Olson et al., 1980; Pohjanvirta et al., 1990; Brewster and Birnbaum, 1988; Durham and Brouwer, 1990). A high percentage of the dose of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF was also found in muscle within the first hour after intravenous exposure because of the large volume of this tissue (Birnbaum et al., 1980; Birnbaum, 1985; Brewster and Birnbaum, 1988). Nevertheless, within several hours the liver, adipose tissue, and skin become the primary sites of disposition, when expressed as percent of administered dose per gram tissue and as percent of dose per organ. Liver, adipose tissue, skin, and thyroid were the only tissues to show an increase in the concentration of 2,3,7,8-TCDD during the initial 4 days after a single intra

peritoneal exposure of rats (Pohjanvirta et al., 1990). In this study, a similar general pattern of disposition was observed in Han/Wistar and Long-Evans rats, which are, respectively, most resistant and susceptible to the acute toxicity of 2,3,7,8-TCDD (Pohjanvirta et al., 1990).

Table 1-4 illustrates the tissue distribution of 2,3,7,8-TCDD in female Wistar rats 7 days after a single subcutaneous exposure (Abraham et al., 1988). This general pattern of distribution is similar to that observed in mice, rats, rhesus monkeys, hamsters, and guinea pigs, where liver and adipose tissue consistently have the highest concentrations of 2,3,7,8-TCDD (Piper et al., 1973; Fries and Marrow, 1975; Rose et al., 1976; Allen et al., 1975; van Miller et al., 1976; Kociba et al., 1978a,b; Gasiewicz et al., 1983b; Manara et al., 1982; Olson et al., 1980; Gasiewicz and Neal, 1979; Birnbaum, 1986; Pohjanvirta et al., 1990; Abraham et al., 1988). A similar pattern of disposition also was observed for 2,3,7,8-TCDF in the guinea pig, rat, C57BL/6J and DBA/2J mouse, and rhesus monkey, with 2,3,7,8-TCDF concentrations highest in liver and adipose tissue (Decad et al., 1981b; Birnbaum et al., 1980, 1981). In summary, there do not appear to be major species or strain differences in the tissue distribution of 2,3,7,8-TCDD and 2,3,7,8-TCDF, with the liver and adipose tissue being the primary disposition sites. For the PCBs, Ness et al. (1994) provide evidence of accumulation of PCB congeners in rat brain. Dams were dosed with Aroclor 1242 and pups from litters were euthanized at weaning. No differences in PCB concentrations between sexes or among brain regions were found, but the different congeners differed from each other in degree of bioaccumulation.

The tissue distribution of the coplanar PCBs and PBBs also appears to be similar to that of 2,3,7,8-TCDD and 2,3,7,8-TCDF. Limited studies in rats and mice found that 3,3′,4,4′-TCB, 3,3′,4,4′-TBB, and 3,3′,4,4′,5,5′-HxBB distributed preferentially to adipose tissue and liver (Clarke et al., 1983, 1984; Millis et al., 1985; Wehler et al., 1989; Clevenger et al., 1989). Lindenau et al. (1994) studied the distribution of PCBs in the reproductive tissues of female rabbits after long-term low-dose exposures. Chlorinated hydrocarbons were found to accumulate especially in oviductal and uterine tissues and in follicular and uterine fluids.

Although the liver and adipose tissue contain the highest concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF, there are some congener-specific differences in the relative tissue distribution of related compounds. 2,3,7,8-TBDD and 1,2,3,7,8-PeCDD disposition in the rat was very similar to that of 2,3,7,8-TCDD (Kedderis et al., 1991a; Wacker et al., 1986). The hepatic concentration of OCDD and 2,3,4,7,8-PeCDF in the rat, however, was approximately 10- to 20-fold greater than that in adipose tissue, which generally contains the second highest levels of these compounds (Birnbaum and Couture, 1988; Norback et al., 1975; Williams et al., 1972; Brewster and Birnbaum, 1987). The tissue distribution of a defined mixture of CDDs and CDFs (28.8 µg CDDs+CDFs/kg bw containing 120 ng 2,3,7,8-TCDD/kg bw) was measured in marmoset monkeys 7 days after a single subcutaneous exposure (Abraham et al., 1990). For most

of the 2,3,7,8-substituted congeners, the highest concentrations were detected in hepatic and adipose tissue, with correspondingly lower values detected in kidney, brain, lung, heart, thymus, or testes. The hepatic and adipose tissue concentrations were similar for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, and 1,2,3,7,8-/1,2,3,4,8-PeCDF. Nonetheless, the hepatic concentrations were approximately tenfold or more greater than those of adipose tissue for 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and OCDF. The lungs and thymus contained higher concentrations of all of these congeners than were detected in kidney, brain, heart, and testes. Unexpectedly, the concentrations of these HxCDDs, HpCDDs, OCDD, and HxCDFs were similar in the adipose tissue, lungs, and thymus. In the case of HpCDFs and OCDF, the concentrations were greater in the lungs than in the adipose tissue. The enhanced disposition of highly chlorinated congeners to the lungs and thymus is of interest and deserves further investigation. For example, it is possible that the high concentration in the lungs could be related to the insolubility of these compounds. Further, Diliberto et al. (1995) report that in mice, the ratio of liver to adipose tissue concentration of 2,3,7,8-TCDD changes with dose. As the dose increases, the adipose (and other nonhepatic tissues) tissue dose/g decreases while it increases in the liver. Section 1.2.5 of this document discusses induction of hepatic cytochrome P-450 1A2 (CYP1A2) as the primary factor responsible for the hepatic sequestration of 2,3,7,8-TCDD and related compounds.

It is also to be expected that exposure to a mixture of these compounds can cause effects in the uptake and distribution. Darnerud et al. (1993) report on the effects of pretreatment of various PCBs on the hepatic uptake of 2,3,7,8-TCDF in mice. After a 4-hour pretreatment, several mono- and di-ortho PCB congeners and Aroclor 1254 increased the hepatic uptake of TCDF. Non-ortho congeners decreased the uptake of TCDF. At longer pretreatment times (48 hours), both non-ortho and mono-ortho PCBs increased the hepatic uptake of TCDF. These studies illustrate the complexity of the pharmacokinetics of mixtures.

Whole-body autoradiography of mice and rats after intravenous administration of [^{14}C]-2,3,7,8-TCDD showed a selective localization of radioactivity in the liver and nasal olfactory mucosa (Appelgren et al., 1983; Gillner et al., 1987). The selective localization of 2,3,7,8-TCDD in the nasal olfactory mucosa was apparently overlooked by other distribution studies that only examined selected organs. Gillner et al. (1987) found no 2,3,7,8-TCDD-derived radioactivity in the olfactory mucosa after solvent extraction of sections, suggesting that 2,3,7,8-TCDD was not covalently bound in this tissue. In addition, Gillner et al. (1987) reported induction of mRNA coding for P-4501A2 (CYP1A2) in the absence of P-4501A1(CYP1A1) induction in olfactory mucosa of rats. The selective distribution of 2,3,7,8-TCDD in the liver and

olfactory mucosa correlates with the tissue-specific localization of CYP1A2, which represents a potential sequestration (binding) protein (see Section 1.2.5). Increases in the incidence of squamous cell carcinoma of nasal turbinates and carcinoma of the liver were observed in rats after a 2-year exposure to 2,3,7,8-TCDD in rat chow (Kociba et al., 1978a); however, this effect was not observed in nasal tissues of mice or rats intubated with 2,3,7,8-TCDD.

Evidence has also been reported that suggests that 2,3,7,8-TCDD uptake and retention by the liver is dependent on the cell type within the liver. Hakansson et al. (1989) found that at 4 days after exposure of rats to 2,3,7,8-TCDD, 60% of the dose distributed to hepatocytes and 12% was retained by stellate cells. Half-lives for 2,3,7,8-TCDD in hepatocytes and stellate cells were calculated to be 13 and 50 days, respectively, suggesting that 2,3,7,8-TCDD is more persistent in nonparenchymal cells. The induction of CYP1A2 in hepatocytes is the primary factor responsible for the hepatic sequestration of 2,3,7,8-TCDD and related compounds (see Section 1.2.5 of this document).

1.2.2.1. *Tissue Distribution in Humans*

Fachetti et al. (1980) reported tissue concentrations of 2,3,7,8-TCDD at levels of 1-2 ng/g in adipose tissue and pancreas, 0.1-0.2 ng/g in the liver, and ≤0.1 ng/g in thyroid, brain, lung, kidney, and blood in a woman who died 7 months after potential exposure to 2,3,7,8-TCDD from the Seveso accident. This pattern of 2,3,7,8-TCDD distribution, however, may not be representative for humans because the woman at the time of death had an adenocarcinoma (which was not considered related to the accident) involving the pancreas, liver, and lung.

Ryan et al. (1985a) examined the distribution of 2,3,7,8-TCDD in two humans at autopsy. They determined on a weight basis that 2,3,7,8-TCDD distributed in descending order to fat (~6 ppt) and liver (~2 ppt), with levels in muscle and kidney below detection; however, 2,3,7,8-TCDD levels compared on a per lipid basis were similar between tissues. These data should be interpreted with caution because only two subjects were examined and one of the subjects was suffering from fatty liver syndrome; therefore, the data cannot be generalized to the entire population.

Poiger and Schlatter (1986) estimated that ~90% of the body burden of 2,3,7,8-TCDD was sequestered in the fat after a volunteer ingested [^{3}H]-2,3,7,8-TCDD in corn oil at a dose of 1.14 ng/kg. During this 135-day study, elevated radioactivity was detected in the blood only during the first 2 days after treatment. The data would be consistent with the high lipid bioconcentration potential of 2,3,7,8-TCDD in humans, as calculated by Geyer et al. (1986) from daily intake assumptions, levels in human adipose tissue, and pharmacokinetic models. Geyer et al. (1986) estimated a BCF of between 104 and 206 for 2,3,7,8-TCDD in human adipose tissue.

In human adipose tissue, levels of 2,3,7,8-TCDD averaging 5-10 ppt have been reported for background populations in St. Louis, Missouri, by Graham et al. (1986), in Atlanta and Utah by Patterson et al. (1986), and in Canada by Ryan et al. (1985b). Sielken (1987) evaluated these data and concluded that the levels of 2,3,7,8-TCDD in human adipose are log-normally distributed and positively correlated with age. Among the observed U.S. background levels of 2,3,7,8-TCDD in human adipose tissue, more than 10% were >12 ppt.

Patterson et al. (1987) developed a high-resolution gas chromatographic/high-resolution mass spectrometric analysis for 2,3,7,8-TCDD in human serum. The arithmetic mean of the individual human serum samples was 47.9 ppt on a whole-weight basis and 7.6 ppt on a lipid-weight basis. Paired human serum and adipose tissue levels of 2,3,7,8-TCDD have been compared by Patterson et al. (1988), Kahn et al. (1988), and Schecter et al. (1990a). All three laboratories reported a high correlation between adipose tissue and serum 2,3,7,8-TCDD levels when the samples were adjusted for total lipid content. This correlation indicates that serum 2,3,7,8-TCDD is a valid estimate of the 2,3,7,8-TCDD concentration in adipose tissue.

Congener-specific partitioning of 2,3,7,8-substituted PCDDs and PCDFs between adipose tissue and plasma lipids has also been reported in a study of 20 Massachusetts Vietnam veterans (Schecter et al., 1990b). The distribution ratio between plasma lipid and adipose tissue increased with chlorine substitution on the PCDDs and PCDFs. Whereas 2,3,7,8-substituted TCDD, TCDF, PeCDD, PeCDF, HxCDD, and HxCDF had a plasma lipid-to-adipose tissue ratio of about 1.0, OCDD had a ratio of 2.0. On the other hand, whole blood PCDDs and PCDFs seem to be found at the same concentrations as in adipose tissue on a lipid basis (Schecter, 1991).

The disposition of 2,3,7,8-substituted PCDDs and PCDFs in human liver and adipose tissue was assessed in a study of 28 people from the Munich area (Thoma et al., 1989; 1990). Table 1-5 summarizes these results, which are expressed both on a lipid and wet-weight basis. The concentrations of PCDDs and PCDFs in adipose tissue and liver are not the same when calculated on a lipid basis. This is in contrast to the high correlation that was reported between adipose tissue and serum TCDD levels when expressed on a lipid-weight basis (Patterson et al., 1988; Kahn et al., 1988; Schecter et al., 1990a). Furthermore, the liver/adipose tissue ratio increased with the higher chlorinated PCDDs and PCDFs. The congener-specific hepatic disposition is also similar to that observed in rats and marmoset monkeys exposed to a complex mixture of PCDDs and PCDFs (Abraham et al., 1989b; Neighbored et al., 1990). Therefore, it is important to consider congener- and tissue-specific differences in disposition of PCDDs and PCDFs when blood levels are used to estimate tissue levels or body burdens.

Schecter et al. (1998a) assessed the disposition of PCDDS, CDFS, and coplanar PBS in the blood, milk, adipose tissue, placenta, and cord blood from five American women. When expressed on a pg/g lipid basis, the mean total TEQs were 11.6, 12.1, 10.5, 5.8, 10.0, and 10.2 in

adipose tissue, predelivery blood, placenta, cord blood, postpartum blood, and breast milk, respectively. The results suggest that CDDs, CDFs, and PCBs, when expressed as total TEQs on a lipid basis, partition to a similar extent between these tissues. Even though 2,3,4,7,8-PeCDF and PCB 126 were lower in cord blood than other tissues, the levels of 2,3,7,8-TCDD were similar in these tissues.

In a study of potentially heavily exposed Vietnam veterans, MMWR (1988) reviewed an Air Force study of Ranch Hand veterans who were either herbicide loaders or herbicide specialists in Vietnam. The mean serum 2,3,7,8-TCDD levels of 147 Ranch Hand personnel was 49 ppt in 1987, based on total lipid weight, while the mean serum level of the 49 controls was 5 ppt. In addition, 79% of the Ranch Hand personnel and 2% of the controls had 2,3,7,8-TCDD levels $\geq$10 ppt. The distribution of 2,3,7,8-TCDD levels in this phase of the Air Force health study indicates that only a small number of Ranch Hand personnel had unusually heavy 2,3,7,8-TCDD exposure. Similar results were obtained by Kahn et al. (1988), who compared 2,3,7,8-TCDD levels in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls (Kahn et al., 1988). This study also examined moderately exposed Vietnam veterans who handled herbicides regularly while in Vietnam. Although this study can distinguish moderately exposed men from others, the data do not address the question of identifying persons whose exposures were relatively low and who constitute the bulk of the population, both military and civilian, that may have been exposed to greater than background levels of 2,3,7,8-TCDD.

1.2.3. Time-Dependent Tissue Distribution

2,3,7,8-TCDD and related compounds exhibit congener-specific disposition, which depends on tissue, species, and time after a given exposure. In general, these compounds are cleared rapidly from the blood and distributed to liver, muscle, skin, adipose tissue, and other tissues within the first hour(s) after exposure. This is followed by redistribution to the liver and adipose tissue, which exhibit increasing tissue concentrations over several days after exposure. Elimination from tissues then occurs at rates that are congener-, tissue-, and species-specific. Thus, the ratio of the concentration of 2,3,7,8-TCDD and related compounds in different tissues (i.e., liver/adipose) may not remain constant over an extended period after a single exposure. Abraham et al. (1988) examined the concentrations of 2,3,7,8-TCDD in liver and adipose tissue of female Wistar rats over a 91-day period after a single subcutaneous exposure at a dose of 300 ng/kg bw (Figure 1-1). The maximum concentration of 2,3,7,8-TCDD in the liver and adipose tissue was reached at 3 and 7 days after exposure, respectively. The liver/adipose tissue concentration ratio does not remain constant over time because the concentration of 2,3,7,8-TCDD decreases more rapidly in the liver than in the adipose tissue. For example, the liver/adipose tissue concentration ratio (for 2,3,7,8-TCDD) was 10.3 at 1 day after exposure and

0.5 at 91 days after exposure (Figure 1-1). Results from other disposition studies also indicate that the ratio of the concentration of 2,3,7,8-TCDD and related compounds in liver, adipose tissue, and other tissues does not remain constant over an extended period after a single exposure (Pohjanvirta et al., 1990; Birnbaum, 1986; Birnbaum et al., 1980; Decad et al., 1981a; Birnbaum and Couture, 1988; Olson et al., 1980; Kedderis et al., 1993a; Brewster and Birnbaum, 1987, 1988; Neubert et al., 1990). This relationship is important in attempting to correlate dose-response data with tissue concentrations of 2,3,7,8-TCDD and related compounds.

In an attempt to maintain constant 2,3,7,8-TCDD levels in tissues to study long-term effects, Krowke et al. (1989) investigated several loading-dose/maintenance-dose exposure regimens. They found that similar liver/adipose tissue concentrations ranging from 5 to 8 could be maintained in rats over a 22-week period with a loading dose of 25 μg/kg followed by weekly maintenance doses of 5 μg/kg.

A large body of data on the tissue concentrations of 2,3,7,8-TCDD and related compounds over time after exposure can be evaluated by estimating congener-specific half-life values for a given tissue and species. Table 1-6 summarizes pharmacokinetic elimination parameters for 2,3,7,8-TCDD and related compounds from major tissue depots. Data from Abraham et al. (1988) (see Figure 1-1) were used to estimate the half-life for 2,3,7,8-TCDD in the liver and adipose tissue of rats (Table 1-6). The decrease in the 2,3,7,8-TCDD concentration in adipose tissue is a linear function in the semilogarithmic plot in Figure 1-1 (log concentration versus time), which indicates apparent first-order elimination kinetics with a half-life of 24.5 days (Table 1-6). Liver tissue exhibits a biphasic (two-component) exponential decay pattern, with a half-life of 11.5 days for the first component (days 10-49) and a half-life of 16.9 days for the second component (days 49-91) (see Figure 1-1 and Table 1-6). Santostefano et al. (1997) reported a similar half-life of approximately 10 days in female Sprague-Dawley rats administered a 10 μg/kg oral dose of 2,3,7,8-TCDD/kg. Results of Abraham et al. (1988) and Lakshmanan et al. (1986) indicate that in the rat, 2,3,7,8-TCDD is more persistent in the adipose tissue than in the liver. This is in contrast to the mouse, where liver and adipose tissue have similar half-lives (Birnbaum, 1986). 2,3,7,8-TCDD is exceptionally persistent in the adipose tissue of the rhesus monkey, with a half-life approximately ten- to fortyfold greater than that observed in the rat and mouse (Bowman et al., 1989). Thus, the relative persistence of 2,3,7,8-TCDD is tissue-specific and exhibits marked interspecies variability.

Most of the pharmacokinetic data on the relative persistence of other congeners in Table 1-6 have been reported in rat studies, which limits interspecies comparisons. Results in the rat suggest that the distribution and elimination of 2,3,7,8-TBDD from tissue are similar to those of 2,3,7,8-TCDD. The most persistent congeners are OCDD, 2,3,4,7,8-PeCDF, and 1,2,3,6,7,8-HxCDF, which distribute almost entirely to the liver. OCDD and 2,3,4,7,8-PeCDF also exhibit

similar elimination kinetics, with a relative half-life in the liver more than twofold greater than that in adipose tissue. The least persistent congeners are 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 3,3′,4,4′-TCB. These congeners exhibit similar elimination kinetics in the rat, with half-lives in the adipose tissue greater than those in liver. The relative tissue distribution of these congeners varies, however, with 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF distributing primarily to the liver while 3,3′,4,4′-TCB distributes predominantly to the adipose tissue.

Viluksela et al. (1997) conducted a subchronic/chronic toxicity study with 1,2,3,4,6,7,8-HpCDD (13 weeks of dosing, 13 week off-dosing) in Sprague-Dawley rats. Liver concentrations during the study provided a hepatic half-life estimate for 1,2,3,4,6,7,8-HpCDD of 237 days in male rats and 314 days in female rats. Under similar exposure conditions, 2,3,7,8-TCDD had half-lives of 12.4 and 15.8 days in male and female rats, respectively. Liver concentrations of CDDs were also assessed in a subchronic/chronic toxicity study in rats administered a mixture of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD orally in corn oil (13 weeks of dosing, 13 weeks of off-dosing) (Viluksela et al., 1998). Average liver half-lives were 14.5, 29.3, 45.6, and 100 days, respectively, in males and 17.8, 38.2, 63.3, and 195.8 days, respectively, in females. When administered individually, 1,2,3,4,7,8-HxCDD had liver half-lives of 36.2 days in males and 55.5 days in females and 1,2,3,7,8-PeCDD had a liver half-life of 36.1 days in male rats. Together, these results indicate that CDDs are more persistent in female rats and that the hepatic half-life of these compounds is similar following exposure to individual CDDs and mixtures of CDDs.

The experimental tissue distribution and elimination data in Table 1-6 were obtained after exposure to a single congener, but real-world exposure to 2,3,7,8-TCDD and related compounds occurs as a complex mixture of congeners. Neubert et al. (1990) examined the persistence of various CDDs and CDFs in hepatic and adipose tissue of male and female marmoset monkeys. Animals received a single subcutaneous exposure to a defined CDD/CDF mixture (total dose of 27.8 µg/kg bw), which contained 0.12 µg 2,3,7,8-TCDD/kg bw. Using the international toxicity equivalency (I-TE) factors (NATO, 1988; U.S. EPA, 1989), the total administered dose corresponded to 0.464 µg I-TE/kg bw. The concentrations of specific congeners in liver and adipose tissue were measured at 1, 6, 16, or 28 weeks after exposure, and elimination constants and half-lives were estimated assuming first-order kinetics (Table 1-7). Data in Table 1-7 were determined from pregnant and nonpregnant female and male marmosets (total of 12 animals), because no obvious differences in tissue concentrations were observed among these groups. All 2,3,7,8-substituted CDDs and CDFs were consistently more persistent in the adipose tissue than in the liver of marmoset monkeys. In general, the persistence in adipose tissue was from ~1.3- to 2.0-fold greater than that in liver, with the exception of 1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF, HpCDFs, and OCDF, which were even more persistent in adipose tissue. For the latter congeners

and OCDD, there was marked variance in half-life values, which may be due to delayed and incomplete absorption of the exceptionally persistent congeners and the relatively short (28 weeks) period of investigation. A significant species difference exists for OCDD and 2,3,4,7,8-PeCDF, which, in contrast to the results in the marmoset monkey, was found to be more persistent in the liver of the rat, with half-lives more than twofold greater than that in adipose tissue (Birnbaum and Couture, 1988; Brewster and Birnbaum, 1987) (see Table 1-6). Further comparison of tissue elimination data in the rat (Table 1-6) and monkey (Table 1-7) indicates that 2,3,7,8-TCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,6,7,8-HxCDF, and 2,3,4,7,8-PeCDF (adipose tissue only) are more persistent in the marmoset monkey than in the rat. The exception to this relationship is 2,3,4,7,8-PeCDF, which is more persistent in rat liver compared to the monkey.

The exposure of marmoset monkeys to a complex mixture of CDDs and CDFs included exposure to both 2,3,7,8- and non-2,3,7,8-substituted congeners (Neubert et al., 1990). One week after exposure to this complex mixture, the non-2,3,7,8-substituted CDDs and CDFs were present in liver and adipose tissue in relatively minor quantities when compared with 2,3,7,8-substituted congeners; however, non-2,3,7,8-substituted compounds represented a considerable percent of the exposure mixture. In this study, none of the non-2,3,7,8-substituted TCDDs, PeCDDs, TCDFs, or PeCDFs could be detected in the liver. Some of the hexa- and hepta-congeners were detected in adipose tissue and liver, but after 1 week, the total amount in the liver was >5% of the administered dose only in the case of 1,2,4,6,8,9-HxCDF. Similar results were obtained in rats after exposure to a defined, complex mixture of CDDs and CDFs (Abraham et al., 1989c). Additional short-term studies in rats provide evidence that the low tissue concentrations of non-2,3,7,8-substituted congeners, measured 1 week after exposure, were the result of rapid elimination, as these congeners were detected at higher levels in the liver 13 to 14 hours after exposure (Abraham et al., 1989d). These results in monkeys and rats are compatible with data from analysis of human tissue samples and milk, in which the non-2,3,7,8-substituted congeners have also not been shown to be present in significant concentrations when compared with the 2,3,7,8-substituted congeners (Schecter et al., 1985, 1986; Ryan, 1986; Rappe et al., 1986; Beck et al., 1987, 1989; Thoma et al., 1989).

Several human studies also show the importance of time dependence and age on tissue distribution within the body. Duarte-Davidson and Jones (1994) reported that in a population in the United Kingdom adipose tissue concentrations of PCBs increased at a slower rate as age increased. The researchers attributed this to the dilution effect caused by the increase in body fat with age. Wolfe et al. (1994) reported similar findings with 2,3,7,8-TCDD. They found significant increases in half-life with increases in percent body fat and with age. As will be discussed in a later section, body burden levels could also be decreasing with time because of reduced exposure levels (Furst et al., 1994).

Other recent studies also show that age is an important factor affecting distribution of these compounds within the body. Pegram et al. (1995) show that the hepatic concentration of 2,3,7,8-TCDD was about 25% greater in young mice than in old mice. No age-related differences in nuclear TCDD concentration was found in the liver fat tissue concentration ratios, adipose tissue, or blood concentrations. In old mice kidney, skin, and muscle concentrations were about twofold higher than in younger mice at a dose of 15 µg/kg. The authors suggest that these age-related differences represent differences in Ah receptor-mediated enzyme induction.

A potential problem of tissue distribution and elimination studies after exposure to a complex mixture of CDDs and CDFs is the possible interaction of the mixture during the uptake and elimination of specific congeners from tissues. A similar hepatic distribution (~25% of dose) and liver/adipose tissue concentrations ratio (~2) for 2,3,7,8-TCDD were observed in rats 7 days after exposure to 2,3,7,8-TCDD (100 ng/kg bw) when the compound was administered alone or in combination with a large amount of other CDDs/CDFs (total 23,222 ng/kg bw) (Abraham et al., 1988, 1989d). This suggests that under these experimental conditions, the tissue distribution of 2,3,7,8-TCDD was not altered when the exposure included a complex mixture of CDDs/CDFs. van den Berg et al. (1989) studied the hepatic disposition and elimination of CDFs administered individually (see Table 1-6) and as mixtures. Co-administration of 1,2,3,7,8- and 2,3,4,7,8-PeCDF resulted in 46% of the dose of 1,2,3,7,8-PeCDF distributing to the liver, while 70% was distributed to the liver after administration of the single-compound (see Table 1-6). Nevertheless, this combined exposure did not alter the rate of elimination of 1,2,3,7,8-PeCDF from the liver. Co-administration of 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HxCDF did not alter the hepatic uptake of either congener or the hepatic elimination of 2,3,4,7,8-PeCDF but increased the hepatic half-life of 1,2,3,6,7,8-HxCDF to 156 days from the single-compound exposure half-life of 73 days (see Table 1-6). However, these values must be considered rough estimates because the experimental period of 42 days was too short to calculate half-lives accurately. Although there are few investigations of potential interactions of mixtures of CDDs and CDFs on the uptake and elimination of individual congeners, the limited available data suggest that exposure to complex mixtures (see Table 1-7) may alter the tissue disposition of individual congeners.

Several studies have investigated potential pharmacokinetic interactions following exposure to two or more PCDDs, PCDFs, or PCBs (DeJongh et al., 1992, 1993a, 1993b, van Birgelen et al., 1996a). Interactive effects on the hepatic disposition of these compounds may in some cases explain, in part, the potentiation and antagonistic effects on CYP1A1/1A2 activities observed with combined exposure to some of these compounds. In the case of 1,2,3,7,8-PeCDD and 2,4,5,2′,4′,5′-HxCB, no pharmacokinetic bases were found to explain the antagonistic effects of the combined exposure on CYP1A1/1A2 activities in mice (DeJongh et al., 1992). In a subsequent study the hepatic disposition of 1,2,3,7,8-PeCDD in mice was increased with

combined exposures to 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, and/or 2,4,5,2′,4′,5′ HxCB (De Jongh et al., 1993b).

De Jongh et al. (1995) report that TCDD co-administration with 2.2',4,4'5,5'-hexachlorobiphenyl (HxCB) in mice results in an increased disposition of TCDD in the liver. At the same time, the results do not show any effect of the TCDD on the HxCB disposition. The distribution of 2,3,7,8-TCDD (0, 0.1, 1.0, or 10 µg/kg) and/or 2.2',4,4',5,5'-hexachlorobiphenyl (PCB 153; 0, 3.58, 35.8, or 358 mg/kg) were investigated alone and in all possible combinations of these doses following oral administration in corn oil to female B6C3F1 mice (van Birgelen et al., 1996b). Coadministration of the low doses of 2,3,7,8-TCDD and PCB 153 had little or no effect on the distribution of either compound. Interactive effects on the pharmacokinetic behavior occurred only at high doses. For example, the hepatic disposition of 2,3,7,8-TCDD increased when it was administered in combination with the highest dose of PCB 153. Although there is evidence that PCDDs, PCDFs, and PCBs may influence each other's pharmacokinetics when administered in mixtures, this area needs investigation.

1.2.4. Dose-Dependent Tissue Distribution

Recent evidence suggests that the tissue distribution of 2,3,7,8-TCDD and related compounds is dose-dependent. Abraham et al. (1988) investigated the distribution of 2,3,7,8-TCDD in liver and adipose tissue of rats 7 days after a single subcutaneous exposure to 2,3,7,8-TCDD at doses of 1-3,000 ng/kg bw. More than 97% of the administered 2,3,7,8-TCDD was absorbed at all doses, with the exception of the 3,000 ng/kg group, where 84% of the dose was absorbed. Figure 1-2 illustrates the dose-dependent disposition of 2,3,7,8-TCDD in liver and adipose tissue (% dose/g) 7 days after exposure. A sharp increase in 2,3,7,8-TCDD concentration in liver was observed at exposure levels >10 ng/kg bw. Disposition in the liver increased from ~11% of the administered dose at an exposure level of 1-10 ng/kg bw to ~37% of the dose at an exposure level of 300 ng/kg bw. The increase in distribution to the liver was accompanied by a dose-related decrease in the concentration of 2,3,7,8-TCDD in the adipose tissue. As a result, the liver/adipose tissue concentration ratio for 2,3,7,8-TCDD at 7 days after exposure increased with increasing doses, starting at an exposure level of 30 ng/kg bw (Table 1-8). Thus, the tissue-specific disposition of 2,3,7,8-TCDD is regulated by a complex relationship, which includes species, time after a given exposure, and dose (see Figures 1-1 and 1-2; Tables 1-6 and 1-7).

Other studies on the tissue disposition of 2,3,7,8-TCDD and related compounds report similar dose-dependent behavior with disproportionately greater concentrations in the liver at high doses compared with low doses. Poiger et al. (1989) observed a dose-related increase in distribution to the liver (% of dose/liver) and an increase in the liver/adipose tissue concentration

ratio for 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF, and 1,2,3,6,7,8-HxCDF in the rat. Kedderis et al. (1991a) also observed a dose-related increase in hepatic disposition (1.27% versus 10.05% of dose/liver) and an increase in the liver/adipose tissue concentration ratio (0.16 versus 2.59) for 2,3,7,8-TBDD at 56 days after exposure at doses of 0.001 and 0.1 µmol/kg bw, respectively. In a related study, pretreatment of mice with 2,3,7,8-TCDD (5 or 15 µg/kg) produced a dose-related, enhanced hepatic accumulation of a subsequent oral dose of 2,3,7,8-TCDD (Curtis et al., 1990). Diliberto et al. (1993b) also observed a dose-dependent tissue distribution of 2,3,7,8-TCDD in female B6C3F1 mice. In all tissues except the liver, the relative percent of the total dose of TCDD decreased while it increased in the liver with higher doses. Similarly, a dose-related increase in hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin was observed after pretreatment of mice with 2,3,7,8-TCDD (Poland et al., 1989a; Leung et al., 1990a). Shen and Olson (1987) also observed an increase in the uptake of 2,3,7,8-TCDD by isolated hepatocytes from 2,3,7,8-TCDD-pretreated mice.

Chronic studies also support dose-dependent alterations in the tissue distribution of these compounds. Kociba et al. (1978a,b) found that female rats maintained on a daily dietary 2,3,7,8-TCDD intake of 100 ng for 2 years had an average 2,3,7,8-TCDD content of 8,100 ppt in fat and 24,000 ppt in the liver. Rats given 10 ng/kg/day had an average of 1,700 ppt 2,3,7,8-TCDD in the fat and 5,100 ppt in the liver. For both of these exposures the liver/adipose tissue concentration ratio of 2,3,7,8-TCDD was ~3. At the lowest dose level of 1 ng/kg/day, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1976) presented evidence that steady state had been reached by <13 weeks of feeding of 2,3,7,8-TCDD.

Other studies do not support the dose-dependent tissue distribution of 2,3,7,8-TCDD and related compounds described above. Rose et al. (1976) reported a lack of a dose-dependent accumulation of [^{14}C]-TCDD in male and female rat liver and adipose tissue following 7, 21, and 49 days of exposure at 0.01, 0.1, or 1.0 µg/kg/day, Monday through Friday. The rates of accumulation of TCDD-derived radioactivity were similar in fat, liver and whole body; however, the concentration in the liver was about fivefold greater than in fat. Brewster and Birnbaum (1987) also observed similar concentrations (% dose/g) of 2,3,4,7,8-PeCDF in liver, adipose tissue, and other tissues 3 days after oral exposure at doses of 0.1, 0.5, or 1.0 µmol/kg bw. These results conflict with the above studies, which support the dose-dependent tissue distribution of these compounds. An explanation for the lack of a dose-dependent accumulation is that all of the doses administered in the studies of Rose et al. (1976) and Brewster and Birnbaum (1987) were inducing hepatic CYP1A2, which is now known to be primarily responsible for the hepatic sequestration of these compounds (see Section 1.2.5).

The dose-dependent tissue distribution of 2,3,7,8-TCDD and related compounds is a critical factor that must be considered in estimating the concentration of these compounds in

human tissues after chronic low-level exposure. This is particularly important because the general human population is exposed to much smaller daily doses (possibly 0.3 pg 2,3,7,8-TCDD/kg/day) than those used in experimental disposition studies. Owing at least partly to the long half-life of 2,3,7,8-TCDD in humans, however, this exposure results in concentrations of 3-18 pg/g in human adipose tissue (Leung et al., 1990b). Similar levels of 2,3,7,8-TCDD in adipose tissue (14 pg/g) were observed in rats 7 days after subcutaneous exposure to 3 ng/kg bw (see Table 1-8) (Abraham et al., 1988). Under these experimental conditions, the liver/adipose tissue 2,3,7,8-TCDD concentration was 0.74. Nonetheless, steady state was definitely not reached under these conditions, and with increasing time after exposure, this ratio may decrease, based on the observation that 2,3,7,8-TCDD was more persistent in adipose tissue than in liver in rats exposed to 300 ng/kg bw (see Figure 1-1 and Table 1-6) (Abraham et al., 1988). Human data on the liver/adipose tissue concentration ratio of 2,3,7,8-TCDD and related compounds are limited, but suggest that the ratio may vary by at least an order of magnitude between individuals. Leung et al. (1990b) observed a geometric mean adipose tissue 2,3,7,8-TCDD concentration of 7.78 ppt in 26 individuals and a concentration in liver at about one-tenth of that in adipose tissue on a whole-weight basis. When measured on a total lipid basis, the concentrations of 2,3,7,8-TCDD in both tissues were approximately the same. Thoma et al. (1990) reported a liver/adipose tissue 2,3,7,8-TCDD concentration on a wet weight basis of 0.14, whereas on a lipid basis the ratio was 2.05 (Table 1-5). Considerable variability in CDD and CDF concentrations in liver and adipose tissues was also observed between individual marmoset monkeys (Neubert et al., 1990), suggesting that individual variability may also contribute to the difficulty in assigning a constant liver/adipose tissue ratio for CDDs and CDFs in humans and nonhuman primates.

1.2.5. Potential Mechanisms for Dose-Dependent Tissue Distribution

The observation that exposure to higher doses of 2,3,7,8-TCDD and related compounds results in a disproportionately greater hepatic concentration of these compounds may be explained by a hepatic binding species that is induced by 2,3,7,8-TCDD and other agonists for the Ah receptor. The studies of Voorman and Aust (1987, 1989) and Poland et al. (1989a,b) provide evidence that this binding species is CYP1A2.

Poland et al. (1989a,b) reported that TCDD and other Ah agonists (2,3,7,8-TCDF, β-naphthoflavone, 3,3′,4,4′,5,5′-hexabromobiphenyl) act through the Ah receptor to increase a liver binding species that increases the hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin (a radiolabeled isosteric analogue of TCDD) in vivo and binding of this radioligand to liver homogenate in vitro. Twenty-four hours after the administration of a non-AHH-inducing dose (1×10^{-10} mol/kg) of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin to C576BL/6J mice, the hepatic concentration of radioactivity was 1%-2% of the administered dose, whereas in mice pretreated

48 hours earlier with an AHH-inducing dose of TCDD (1×10^{-7} mol/kg), the hepatic accumulation of radiolabel was 25%-30% of that administered. A similar, though less dramatic, effect was observed in vitro, with liver homogenate from TCDD-treated mice binding about four times more [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin than homogenate from control mice. The administration of TCDD to C57BL/6J mice produced a dose-related stimulation of in vivo hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin, increased binding of radioligand to liver homogenate, and induction of hepatic activity, with an ED_{50} ranging from 1.5 to 4.0×10^{-9} mol/kg. In congenic C57BL/6J (Ah^d/Ah^d) mice, which express the lower affinity Ah receptor, the ED_{50} values for all three responses were shifted to doses that were about tenfold higher. The observed effects on hepatic disposition were tissue-specific, with no remarkable dispositional changes being observed in kidney, lung, spleen, small intestine, or muscle. This is significant in that TCDD and other agonists for the Ah receptor induce CYP1A1 in liver and other tissues, whereas CYP1A2 is apparently inducible only in liver (Tuteja et al., 1985; Gillner et al. 1987). Furthermore, the changes in hepatic disposition were not species-specific; similar responses were observed in guinea pigs, rats, mice, and hamsters (Poland et al., 1989a).

The following evidence reported by Poland et al. (1989b) supports the hypothesis that the TCDD-inducible hepatic binding protein is CYP1A2: the TCDD-induced hepatic binding species was found predominantly in the microsomal fraction and was inactivated by heating at 60°C, trypsin, and mercurials; the TCDD-induced hepatic binding species was specific for the liver, with a large pool size (B_{max} of 22 ± 5 nmol/g liver); the major microsomal binding species covalently labeled with the photoaffinity ligand [^{125}I]-2-iodo-3-azido-7,8-dibromodibenzo-p-dioxin migrates with that immunochemically stained with polyclonal antiserum, which binds to CYP1A2.

One observation of Poland et al. (1989a,b) does not support the hypothesis that the TCDD-inducible hepatic protein is CYP1A2. These investigators found that dietary administration of isosafrole did not stimulate hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin or the in vitro binding of this ligand to liver homogenate. Isosafrole is not an agonist for the Ah receptor, but it selectively induces CYP1A2 (Ryan et al., 1980). Poland et al. (1989a,b) suggest that this may be attributable to the high affinity binding of an isosafrole metabolite to the protein, which might inhibit the binding of [^{125}I]-iodo-3,7,8-trichlorodibenzo-p-dioxin to CYP1A2 at or near the active site of the enzyme. This does not explain why TCDD, which also has high affinity for CYP1A2, cannot displace some of the isosafrole metabolite from the protein, which should produce enhanced hepatic disposition of TCDD.

A more recent study used pretreatment with isosafrole to investigate the role of CYP1A2 in the hepatic disposition of 2,3,7,8-TCDD (Kedderis et al., 1993a). Although isosafrole is an inducer of CYP1A2, it also has high affinity for the protein and thus is a selective inhibitor of

CYP1A2. A greater than threefold decrease in the hepatic disposition of TCDD in isosafrole-pretreated rats supports the conclusion that TCDD is bound to CYP1A2 in the liver.

Olson et al. (1994) have reported the results of in-vitro studies with isolated hepatocytes and liver slices of rats pretreated with TCDD. Pretreatment increased the uptake of TCDD but not TCDF. There was an increase in both CYP1A1 and CYP1A2. The authors interpret these results as consistent with CYP1A2 being a hepatic binding protein for TCDD but not TCDF.

Voorman and Aust (1987, 1989) support further the hypothesis that CYP1A2 is the TCDD-inducible hepatic binding species. These investigators found that 3,3′,4,4′,5,5′-HxBB, an agonist for the Ah receptor, was associated only with CYP1A2 through the immunoprecipitation of CYP1A1 and 1A2, which were induced in 3,3′,4,4′,5,5′-HxBB treated rats. In addition, they found that 3,3′,4,4′,5,5′-HxBB inhibited estradiol 2-hydroxylase activity of purified CYP1A2. A similar association of PAHs with immunoprecipitated CYP1A2 was observed for other agonists for the Ah receptor, including 2,3,7,8-TCDD, 3,3′,4,4′-TCB, 3,3′,4,4′,5-PeCB, and 3,3′,4,4′,5,5′-HxCB. The association of 2,3,7,8-TCDD with CYP1A2 occurred within 2 minutes, with maximum inhibition of estradiol 2-hydroxylase occurring at a concentration comparable to the concentration of the enzyme (50 nm). CYP1A2 was inhibited (complexed) by 2,3,7,8-TCDD with nearly 1:1 stoichiometry, and the K_i for 2,3,7,8-TCDD was calculated to be 8 nM. Therefore, 2,3,7,8-TCDD can be considered a higher binding inhibitor of CYP1A2.

Santostefano et al. (1996) assessed the subcellular and tissue-specific disposition of 2,3,7,8-TCDD in rats and mice. 2,3,7,8-TCDD was equally distributed between the hepatic P9 (mitochondrial, lysosomal, and nuclear) and S9 (cytosol and microsomal) fractions, with the microsomal fraction retaining the 2,3,7,8-TCDD present in the S9 fraction. In contrast, 2,3,7,8-TCDD was retained in the P9 fractions of lung and liver at all doses tested. The lack of pulmonary or renal sequestration coupled with the lack of localization of 2,3,7,8-TCDD to pulmonary and renal microsomes supports the role of CYP1A2 as a hepatic microsomal binding protein involved in the hepatic sequestration of 2,3,7,8-TCDD. Recently, Santostefano et al. (1999) assessed the intralobular hepatic distribution of 2,3,7,8-TCDD in rats and observed that centrilobular hepatocytes had a 2.7- to 4.5-fold higher concentration of 2,3,7,8-TCDD than did periportal hepatocytes. The enhanced centrilobular distribution of 2,3,7,8-TCDD was associated with elevated CYP1A2-mediated MROD activity and CYP1A2 mRNA in centrilobular hepatocytes.

The TCDD-induced binding species was found to have an apparent equilibrium dissociation constant, K_D, for [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin of 56 ± 16 nM, and a pool size, B_{max}, of 22±5 nmol/g of liver in C57BL/6J mice (Poland et al., 1989b). The induced microsomal binding species has an affinity about 10^4 times less than the Ah receptor but a pool size that is $\sim 2 \times 10^3$ greater. Thus, agonists for the Ah receptor may significantly affect their

disposition through a dose-related enhancement of hepatic uptake, which should correlate with induction of CYP1A2.

More recently, Diliberto et al. (1997) used transgenic mice lacking the CYP1A2 gene to study the influence of CYP1A2 in the hepatic sequestration and distribution of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, and PCB 153 (2,2',4,4',5,5'-HexCB), a non-dioxin-like PCB. The liver/fat concentration ratios of these compounds in the parental lineage (C57BL6N and 129/Sv) were approximately 3.6, 18, and 0.07, respectively, indicating a high degree of hepatic sequestration for 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF. Under identical exposure conditions, the 1A2 knockout mice had liver/fat concentration ratios of 0.17, 0.34, and 0.10, respectively. Thus, in the absence of the CYP1A2 gene, mice exhibited no hepatic sequestration of these compounds. This study and a related study in CYP1A2 knockout mice by Diliberto et al. (1999) provide direct confirmation of the hypothesis that CYP1A2 is the dioxin-inducible hepatic binding protein responsible for the hepatic sequestration of 2,3,7,8-TCDD and related compounds, such as 2,3,4,7,8-PeCDF.

The disposition and pharmacokinetics of 2,2′,4,4′,5,5′-HxCB and -HxBB have been investigated in several species (Tuey and Matthews, 1980; Lutz et al., 1984). These lipophilic compounds are similar to 2,3,7,8-TCDD in that they are slowly metabolized and that metabolism is required for urinary and biliary elimination; however, they do not exhibit dioxin-like activity. 2,2′,4,4′,5,5′-HxCB and -HxBB distribute primarily to adipose tissue, with partition coefficients (tissue/blood ratio) ranging from 300 to 500 in the mouse, rat, monkey, dog, and human. The liver is not a major site for the disposition of 2,2′,4,4′,5,5′-HxCB and -HxBB, in contrast to 2,3,7,8-TCDD and related compounds. Partition coefficients in the liver range from 10 to 30 in these species. 2,2′,4,4′,5,5′-HxCB and -HxBB do not induce hepatic CYP1A1 or 1A2 and do not exhibit dioxin-like activity. The lack of induction of hepatic CYP1A2 may explain the lack of a dose-dependent hepatic disposition of these compounds.

Kedderis et al. (1991b) assessed the dose-response relationship for the induction of hepatic CYP1A1 and 1A2 in male Fischer 344 rats exposed to 2,3,7,8-TBDD at doses as low as 0.1 nmol/kg. They reported that induction of P-4501A2 by 2,3,7,8-TBDD appeared to be a more sensitive response than P-4501A1 induction over the dose range studied. In addition, comparison of hepatic P-4501A2 levels and liver:adipose tissue concentration ratios suggested that induction of P-4501A2 alone would not directly account for the preferential hepatic accumulation of 2,3,7,8-TBDD and that additional factors must be involved. One explanation may be that at low 2,3,7,8-TBDD concentrations, endogenous substrates bind to CYP1A2, not allowing 2,3,7,8-TBDD to be sequestered by the protein (Kedderis et al., 1993b). At higher 2,3,7,8-TBDD concentrations, new protein is formed and 2,3,7,8-TBDD can compete for binding to CYP1A2,

resulting in the increased hepatic disposition observed at higher exposures of 2,3,7,8-TBDD (Kedderis et al., 1991b).

The structure-activity relationship for the disposition and hepatic sequestration of CDDs, CDFs, and PCBs was investigated by DeVito et al. (1998). Female B6C3F1 mice were treated for 13 weeks with different oral doses of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TBDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, OCDF, PCB126 (3,3',4,4',5-PeCB), PCB169 (3,3'4,4',5,5'HxCB), PCB105 (2,3,3',4,4' PeCB), PCB118 (2,3',4,4',5-PeCB), and PCB156 (2,3,3',4,4',5-HxCB). All of these compounds exhibited dose-dependent increases in the liver/fat concentration except PCBs 105, 118, and 156. 4-PeCDF, PeCDD, OCDF, TCDF, and PCB126 were sequestered in hepatic tissue to a greater extent than was 2,3,7,8-TCDD. Together, the results support the presence of an inducible protein (CYP1A2) and the congener-specific binding of some dioxin-like compounds to this hepatic sequestration protein.

Other factors may also regulate the intracellular distribution of 2,3,7,8-TCDD and related compounds. The possible role of hepatic lipoproteins as intracellular carriers in the transport of 2,3,7,8-TCDD has been assessed by in vitro and in vivo studies (Souès et al., 1989a,b). 2,3,7,8-TCDD and 2,3,7,8-TCDF were bound to lipoproteins in mouse and rat liver, which subsequently underwent rapid and pronounced degradative processing, possibly catalyzed by lipoprotein lipase, to heavier entities. The in vitro incubation of 2,3,7,8-TCDD-lipoprotein complex with separated Ah receptor demonstrated that a passive transfer occurred. The authors suggest a carrier role for lipoproteins in the intracellular transport of 2,3,7,8-TCDD and related compounds.

1.3. METABOLISM AND EXCRETION

Although early in vivo and in vitro investigations were unable to detect the metabolism of 2,3,7,8-TCDD (Vinopal and Casida, 1973; van Miller et al., 1976), there is evidence that a wide range of mammalian and aquatic species are capable of biotransforming 2,3,7,8-TCDD to polar metabolites (Ramsey et al., 1979, 1982; Poiger and Schlatter, 1979; Olson et al., 1980; Olson, 1986; Gasiewicz et al., 1983a; Poiger et al., 1982; Sijm et al., 1990; Kleeman et al., 1986a,b, 1988). Although metabolites of 2,3,7,8-TCDD have not been directly identified in humans, recent data regarding feces samples from humans in a self-dosing experiment suggest that humans can metabolize 2,3,7,8-TCDD (Wendling et al., 1990).

Table 1-9 summarizes data on the metabolism and excretion of 2,3,7,8-TCDD and related compounds after exposure to a single radiolabeled congener. Investigations of 2,3,7,8-TCDD in rats, mice, guinea pigs, and hamsters found that >90% of the radiolabeled material excreted in urine and bile represented polar metabolites. Similar results were also observed for other congeners (see Table 1-9), with the exception of OCDD; however, studies were often limited to the rat. OCDD is apparently not metabolized by the rat, or metabolized to a very minimal extent

(Birnbaum and Couture, 1988). For all of the congeners in Table 1-9, essentially all of the CDD, BDD, CDF, or PCB-derived radioactivity in liver, adipose tissue, and other tissues represented parent compound, suggesting that metabolites of these compounds were readily excreted. Thus, with the exception of OCDD, the metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and therefore plays a major role in regulating the rate of excretion of these compounds. In addition, direct intestinal excretion of parent compound is another route for excretion of 2,3,7,8-TCDD and related compounds that is not regulated by metabolism.

1.3.1. Structure of Metabolites

Several metabolites of 2,3,7,8-TCDD have recently been identified. Sawahata et al. (1982) investigated the in vitro metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The major product was deconjugated with β-glucuronidase, derivatized with diazomethane, and separated into two compounds by high-performance liquid chromatography. These metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin. Poiger et al. (1982) identified six metabolites in the bile of dogs that were given a lethal dose of [^{3}H]-2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin; however, 3,7,8-trichloro-3-hydroxydibenzo-p-dioxin and 1,2-dichloro-4,5-hydroxybenzene were identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichlorohydroxydibenzo-p-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether. Poiger and Buser (1984) reported differences in the relative amounts of various 2,3,7,8-TCDD metabolites in dog and rat bile. Trichlorodihydroxy-dibenzo-p-dioxin and tetrachlorodihydroxydiphenyl ether appear to be major metabolites in rat bile. Furthermore, conjugates, presumably glucuronides, were formed in the rat but not in the dog. The investigators also observed a generally higher metabolism rate of 2,3,7,8-TCDD in the dog. This finding is in good agreement with the unique ability of the dog to readily metabolize persistent PCBs such as 2,4,5,2′,4′,5′-HxCB (Sipes et al., 1982).

Biliary metabolites of 2,3,7,8-TCDF have been investigated by Poiger et al. (1984); however, unequivocal structure assignment of the metabolites could not be made using gas chromatography/mass spectroscopy. With the use of synthetic standards and GC/MS, Burka et al. (1990) identified 4-hydroxy-2,3,7,8-TCDF and 3-hydroxy-2,7,8-TCDF as major biliary metabolites of 2,3,7,8-TCDF in rats. Small amounts of 3-hydroxy-2,4,7,8-TCDF and 2,2′-dihydroxy-4,4′,5,5′-TCB were also detected. 4-Hydroxy-2,3,7,8-TCDF was also the major TCDF metabolite formed by hepatic microsomes from TCDD-pretreated rats (Tai et al., 1993). This suggests that the preferred site of metabolism of 2,3,7,8-TCDF is near the furan oxygen,

with oxygenation at C4 predominating over C3. The authors speculate that epoxidation of the C4-C4a bond or the C3-C4 bond could lead to formation of the above metabolites. The results of Burka et al. (1990) and Sawahata et al. (1982) suggest that oxygenation of the unsubstituted carbon nearest the bridging oxygen in both 2,3,7,8-TCDF and 2,3,7,8-TCDD is the major route of metabolism of these compounds in the rat. Furthermore, data on the rate of elimination of these compounds summarized in Tables 1-6 and 1-8 indicate that this reaction occurs at a faster rate for the furan, since the rate of urinary and biliary elimination and resulting persistence of these compounds depend on metabolism.

Data summarized in Tables 1-6 and 1-9 indicate that 1,2,3,7,8-PeCDF is metabolized and eliminated at a greater rate than 2,3,4,7,8-PeCDF. The preference for oxygenation at C4 in 2,3,7,8-TCDF offers an explanation for the observation that 2,3,4,7,8-PeCDF is metabolized at a much slower rate than 1,2,3,7,8-PeCDF because one of the preferred sites for metabolism is blocked in the 2,3,4,7,8-substituted compound. The rate of metabolism of these compounds and their resulting relative persistence in rodents correlate with analysis of human tissues from the Yusho cohort, where the relative concentrations were 2,3,4,7,8-PeCDF > 1,2,3,7,8-PeCDF > 2,3,7,8-TCDF (Masuda et al., 1985).

Pluess et al. (1987) investigated the structure of 1,2,3,7,8-PeCDF metabolites in rat bile. A dihydroxy-tetra-CDF was identified as the major metabolite. The authors propose that this compound could be formed either via further oxidation of the hydroxy-tetra-CDF or possibly via hydrolytic dechlorination of a hydroxy-penta-CDF. Minor metabolites include dihydroxy-tri-CDF, hydroxy-tetra-CDF, and hydroxy-penta-CPF.

Pluess et al. (1987) also investigated the metabolites of 2,3,4,7,8-PeCDF in rat bile. A total of 10 metabolites were detected, with dihydroxy-penta-CB and hydroxy-penta-CDF representing the major metabolites. The biphenyl metabolite indicates that cleavage of the ether bridge of the furan is an important pathway for metabolism of this congener. Other less abundant metabolites of 2,3,4,7,8-PeCDF include hydroxy-tetra-CDF, dihydroxy-tri-CDF, dihydroxy-tetra-CDF, and thio-tetra-CDF. Sulfur-containing metabolites were also identified as minor metabolites of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF in rats (Kuroki et al., 1990). These sulfur-containing metabolites probably arise from CDF-glutathione conjugates.

In another study, a dihydroxy-PeCDF was identified as the only detectable biliary metabolite of 1,2,3,6,7,8-HxCDF, while no metabolites of 1,2,3,4,6,7,8-HpCDF were detected in the bile of rats treated with this congener (Poiger et al., 1989).

Several in vivo and in vitro studies have investigated the metabolism of 3,3′,4,4′-TCB. Rat feces were found to contain 5-hydroxy-3,3′,4,4′-TCB and 4-hydroxy-3,3′,4′,5-TCB as major metabolites (Yoshimura et al., 1987) and 2,5-dihydroxy-3,3′,4,4′-TCB, 4,4′-dihydroxy-3,3′,5,5′-

TCB, 5,6-dihydroxy-3,3′,4,4′-TCB, 4-hydroxy-3,3′,4-TCB, and 4-hydroxy-4′,5′-epoxy-3,3′,4′,5-TCB as minor metabolites (Koga et al., 1989).

Mouse feces were found to contain 5-hydroxy- and 6-hydroxy-3,3′,4,4′-TCB and 4-hydroxy-3,3′,4′,5-TCB, whereas urine contained 2-hydroxy-3,3′,4,4′-TCB in addition to these metabolites (Wehler et al., 1989). 3,3′,4,4′-TCB was the major compound present in mouse liver and a minor portion was due to 4-hydroxy-3,3′,4,4′-TCB (Wehler et al., 1989). Darnerud et al. (1986) found 2-hydroxy-3,3′,4,4′-TCB and methylsulphonyl-TCB as major metabolites in the mouse fetus. Sulfur-containing metabolites of non-co-planar, nondioxin-like PCBs have also been reported to accumulate in the bronchial mucosa and uterine luminal fluid of mice (Bergman et al., 1979; Brandt et al., 1982) and in human lung, liver, and adipose tissue (Haraguchi et al., 1986, 1989). PCB methyl sulfones are stable lipophilic metabolites formed by the mercapturic acid pathway. The toxicological significance of these metabolites of nondioxin-like PCBs remains generally unknown.

1.3.2. Toxicity of Metabolites

The discussion above indicates that metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and thus plays a major role in regulating the rate of excretion of these compounds. At present, metabolism is also generally considered a detoxification process.

Data on the metabolism of 2,3,7,8-TCDD suggest that reactive epoxide intermediates may be formed. Poland and Glover (1970) investigated the in vivo binding of [1,6-^{3}H]-2,3,7,8-TCDD-derived radioactivity to rat hepatic macromolecules and found maximum levels equivalent to 60 pmol of 2,3,7,8-TCDD/mol of nucleotide in RNA and 6 pmol of 2,3,7,8-TCDD/mol of nucleotide in DNA. This corresponds to one 2,3,7,8-TCDD-DNA adduct per 35 cells. These investigators suggest that it is unlikely that 2,3,7,8-TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation. Further studies of 2,3,7,8-TCDD and related compounds are needed to confirm these results and assess the relationship between covalent binding and the short- and long-term toxicity of these compounds.

Weber et al. (1982a) investigated the toxicity of 2,3,7,8-TCDD metabolites by administering extracts of bile from 2,3,7,8-TCDD-treated dogs to male guinea pigs in single oral doses equivalent to 0.6, 6.0, and 60 µg/kg of parent compound. Other groups of guinea pigs were given bile extract from untreated dogs or 2,3,7,8-TCDD itself. A comparison of the mortality data at 5 weeks after dosing indicated that the acute toxicity of 2,3,7,8-TCDD to guinea pigs was at least 100 times higher than the acute toxicity of its metabolites.

Mason and Safe (1986) synthesized 2-hydroxy-3,7,8-TCDD and 2-hydroxy-1,3,7,8-TCDD, which are metabolites of 2,3,7,8-TCDD, and assessed the toxicity of these compounds in

male Wistar rats. The compounds produced no significant effect on body weight gain and thymus, liver, or spleen weights after exposure to a dose of ≤5,000 µg/kg bw. 2-Hydroxy-3,7,8-TCDD induced hepatic microsomal AHH, EROD, and 4-chlorobiphenylhydroxylase activity at exposures of 1,000 and 5,000 µg/kg bw, whereas 2-hydroxy-1,3,7,8-TCDD was inactive as an inducer. Thus, while 2-hydroxy-3,7,8-TCDD has dioxin-like activity as an inducer of the hepatic monooxygenase system, the potency of the metabolite is more than three orders of magnitude less than that of 2,3,7,8-TCDD. Furthermore, results are consistent with the expected rapid conjugation and excretion of these 2,3,7,8-TCDD metabolites (Weber et al., 1982b).

Metabolism of co-planar PCBs and PBBs also appears to be a detoxification process. 5-Hydroxy-3,3′,4,4′-TCB and 4-hydroxy-3,3′,4′,5-TCB did not produce liver hypertrophy, induction of hepatic AHH or DT-diaphorase activities, or thymus atrophy (Yoshimura et al., 1987). Thus, monohydroxy metabolites of 3,3′,4,4′-TCB are much less toxic than the parent compound. Further evidence for metabolism as a detoxification process comes from comparison of the metabolism and toxicity of two co-planar PBBs. Millis et al. (1985) found that 3,3′,4,4′,5,5′-HxBB exhibited greater toxic potency in rats than 3,3′,4,4′-TBB, even though 3,3′,4,4′-TBB had about a 10-fold greater affinity for the Ah receptor. Although receptor binding affinities imply that 3,3′4,4′-TBB should be more toxic than 3,3′,4,4′5,5′-HxBB, it was less toxic than the HxBB because 3,3′,4,4′-TBB was metabolized at a much greater rate than 3,3′,4,4′,5,5′-HxBB. In addition to supporting metabolism as a detoxification process, the results of Millis et al. (1985) also suggest that receptor binding and in vitro AHH induction do not accurately reflect toxicity for PAHs, which are more readily metabolized, presumably because continued occupation of the receptor is required for toxicity.

Structure-activity studies of 2,3,7,8-TCDD and related compounds support the widely accepted principle that this parent compound is the active species. The relative lack of activity of readily excreted monohydroxylated metabolites of 2,3,7,8-TCDD and 3,3′4,4′-TCB suggests that metabolism is a detoxification process necessary for the biliary and urinary excretion of these compounds. This concept has also been generally applied to 2,3,7,8-TCDD-related compounds, although data are lacking on the structure and toxicity of metabolites of other CDDs, BDDs, CDFs, BDFs, PCBs, and PBBs.

It is possible that low levels of unextractable and unidentified metabolites may contribute to one or more of the toxic responses of 2,3,7,8-TCDD and related compounds. Further studies on the nature of the biotransformation products of these compounds will help address this uncertainty.

1.3.3. Autoinduction of Metabolism

Accurate rate constants for metabolism are important in developing pharmacokinetic models that describe the disposition of 2,3,7,8-TCDD and related compounds. Metabolism plays a major role in regulating the excretion and relative persistence of these compounds because metabolism is required for urinary and biliary excretion. Although the relative rate of metabolism of 2,3,7,8-TCDD and related compounds can be estimated from tissue and excretion half-life data (see Tables 1-6 and 1-9), other factors such as relative body composition, hepatic and extrahepatic binding proteins, and direct intestinal elimination of the parent compound can also regulate the excretion of 2,3,7,8-TCDD and related compounds. Therefore, in vivo disposition data (see Tables 1-6 and 1-9) provide only a limited approximation of the relative rate of metabolism of a specific congener in a given species. In vivo disposition data were also obtained at exposures that were associated with induction of CYP1A1 and 1A2 and other potentially adverse responses that could alter metabolism and disposition. Therefore, it may not be appropriate to directly extrapolate these data to predict the pharmacokinetics at low levels of exposure. Low-dose extrapolations can be assisted by assessments of the potential for autoinduction of metabolism that may occur at exposures associated with enzyme induction. Characterization of the dose-dependent disposition of 2,3,7,8-TCDD and related compounds is particularly important in the extrapolation of high-exposure animal data to low-exposure human data.

The excretion of metabolites of 2,3,7,8-TCDD and related compounds into bile represents a direct means for estimating the rate of metabolism because biliary elimination depends on metabolism and is the major route for excretion of these compounds. The rate of metabolism of CDFs was estimated from the relative abundance of metabolites in rat bile (Poiger et al., 1989). The rates of biotransformation of 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, and 1,2,3,6,7,8-HxCDF were characterized as fairly high, moderate, low, and very low, respectively. Kedderis et al. (1991b, 1993a) observed 10% of the dose of [^{3}H]-2,3,7,8-TBDD excreted in bile 5 hours after intravenous administration of 1 nmol/kg to male Fischer 344 rats. All biliary radioactivity was attributable to metabolites. This rate of elimination is similar to the fecal excretion (~8% of the dose) 24 hours after intravenous administration of 1 nmol/kg [^{3}H]-2,3,7,8-TBDD (Kedderis et al., 1991a) and reflects the effect of intravenous bolus versus oral administration on distribution and elimination. The large percent dose excreted within the first few days may also be due to a rapidly excreted impurity in the radiolabeled 2,3,7,8-TBDD (Kedderis et al., 1993a). To assess the ability of 2,3,7,8-TCDD and 2,3,7,8-TBDD to induce their own metabolism (biliary elimination), rats were pretreated with 100 nmol/kg, per of, of each compound 3 days prior to intravenous injection of 1 nmol/kg of the respective [^{3}H]-congeners. Biliary excretion of the radiolabeled dose was quantitatively and qualitatively unaffected by

pretreatment, despite a twofold increase in hepatic levels of [^{3}H] in the pretreated animals and significant induction of CYP1A1 and 1A2 (Kedderis et al., 1991b). Therefore, under these conditions, autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism did not occur in the rat in vivo at doses that elicited enhanced hepatic uptake. Similarly, Curtis et al. (1990) observed no change, or even an apparent decrease, in gastrointestinal contents and fecal elimination of TCDD equivalents in pretreated versus naive mice 24 hours after oral administration of [^{14}C]-2,3,7,8-TCDD, despite significantly enhanced levels of 2,3,7,8-TCDD in the livers of pretreated mice.

Although the above studies suggest that autoinduction of 2,3,7,8-TCDD metabolism does not occur, other results indicate that metabolism may be induced under certain conditions. Poiger and Buser (1984) observed a small yet significant increase in biliary excretion over a 72-hour period, with pretreated rats (10 µg/kg, intraperitoneal) excreting 9.7±1.9% of the radiolabeled dose of 2,3,7,8-TCDD (200-300 µg/kg, per of) compared with 7.0±0.9% excreted by naive animals. In addition to being small changes, these results were obtained using a dose of 2,3,7,8-TCDD in excess of the LD_{50} in the rat. Poiger and Schlatter (1985) examined the influence of pretreatment with phenobarbital and 2,3,7,8-TCDD on the biliary excretion of [^{3}H]-2,3,7,8-TCDD metabolites in a dog given a single oral dose of the [^{3}H]-congener (31 or 33.8 ng/kg). Without pretreatment, 24.5% of the absorbed dose was excreted in the bile within 110 hours. Phenobarbital did not alter this rate, whereas pretreatment with 2,3,7,8-TCDD (10 µg/kg) 9 days earlier resulted in a doubling of the amount of metabolites excreted in bile (47.4%). Although this observation is limited to one dog and requires further investigation, the results suggest that significant autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion may occur in the dog. Nonetheless, the small increase in metabolism and biliary excretion of 2,3,7,8-TCDD in the rat observed by Poiger and Buser (1984) and the negative results of Kedderis et al. (1991b; 1993a) and Curtis et al. (1990) suggest that autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion in the rat may not occur or occurs to an extent that is not biologically relevant.

More recently, Jackson et al. (1998) investigated the effects of age, sex, and pretreatments with phenobarbital, dexamethasone, and 1-aminobezotriazole (a CYP450 inhibitor) to modulate the rate of metabolism-dependent biliary elimination of 2,3,7,8-TCDD in F344 rats. Biliary excretion of 2,3,7,8-TCDD-derived radioactivity was measured over a 6-hour period following iv administration. Male adult and juvenile rats and female juvenile rats excreted from 0.63% to 0.68% of the administered dose, while adult females, male senescent, and male adults pretreated with the above drugs excreted from 0.28% to 0.45% of the dose of 2,3,7,8-TCDD in bile. The results suggest some variability in the metabolism-dependent biliary excretion of 2,3,7,8-TCDD-derived radioactivity; however, the differences between groups were not great.

Limited data suggest that autoinduction of metabolism and biliary excretion does occur for CDFs. Pretreatment of rats with 2,3,7,8-TCDF (1.0 µmol/kg, 3 days earlier) significantly increased the biliary excretion of a subsequent dose of [^{14}C]-2,3,7,8-TCDF (McKinley et al., 1993). The naive rats excreted 5.69±2.35% of the dose over the initial 8 hours, while the pretreated rats excreted 13.18±3.15% of the [^{14}C]-2,3,7,8-TCDF. Similarly, pretreatment of rats with 2,3,4,7,8-PeCDF (500 µg/kg, per of, 3 days earlier) resulted in a twofold increase in the biliary elimination of a subsequent dose of [^{14}C]-2,3,4,7,8-PeCDF (Brewster and Birnbaum, 1987). These results suggest that pretreatment with 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF induces the metabolism of these congeners.

3,3′,4,4′-TCB and 3,3′,4,4′-TBB appear to be metabolized by a 3-methylcholanthrene-inducible form of hepatic CYP(1A1 or 1A2), which is also induced by 3,3′,4,4′-TCB (Shimada and Sawabe, 1983; Mills et al., 1985; McKinley et al., 1993). This suggests that these compounds can induce their own rate of metabolism and subsequent excretion.

Isolated hepatocytes in suspension culture have been demonstrated to provide a useful in vitro system for studying the hepatic metabolism of 2,3,7,8-TCDD under the same conditions in species that have a wide range of sensitivity to the compound (Olson et al., 1981). The in vitro rate of metabolism of 2,3,7,8-TCDD in guinea pig, rat, C57BL/6J mouse, DBA/2J mouse, and hamster hepatocytes was estimated to be 0.2, 1.2, 1.1, 0.9, and 1.2 pmol/mg protein/hour, respectively (Wroblewski and Olson, 1985, 1988; Shen and Olson, 1987). These results indicate that 2,3,7,8-TCDD is metabolized by the guinea pig liver at a rate about fivefold less than that observed for the rat, mouse, and hamster. The limited ability of the guinea pig to metabolize 2,3,7,8-TCDD can explain the limited excretion of 2,3,7,8-TCDD metabolites in feces, which represents the major route for 2,3,7,8-TCDD excretion (Olson, 1986). In addition, the limited metabolism in the guinea pig may partly explain the relatively long excretion half-life for 2,3,7,8-TCDD in the guinea pig and may contribute to the remarkable sensitivity of the guinea pig to the acute toxicity of this agent (Olson, 1986).

Isolated hepatocytes in suspension culture have been used as an in vitro system for studying the autoinduction of metabolism of 2,3,7,8-TCDD and related compounds. Wroblewski and Olson (1988) investigated the metabolism of [^{14}C]-2,3,7,8-TCDD (2.2 µM) in hepatocytes isolated from untreated 2,3,7,8-TCDD-, 3-MC-, isosafrole-, and phenobarbital-pretreated rats and hamsters. In both species, 2,3,7,8-TCDD and 3-MC pretreatments elevated the rate of 2,3,7,8-TCDD metabolism by five- to sixfold, while phenobarbital pretreatment had no effect. Isosafrole produced a 1.8- to 2.5-fold increase in metabolism. These in vitro results at a high substrate concentration (2.2 µM) indicate that 2,3,7,8-TCDD can induce its own rate of metabolism in the rat and hamster. In contrast, 2,3,7,8-TCDD was not able to induce its own rate of metabolism in guinea pig and mouse hepatocytes (Wroblewski and Olson, 1985; Shen and Olson, 1987).

Together, these results indicate that 2,3,7,8-TCDD is metabolized in the liver by a 2,3,7,8-TCDD-inducible enzyme, which is expressed in the rat and hamster but not in the guinea pig and mouse. More recently, the kinetics of 2,3,7,8-TCDD metabolism were investigated in isolated rat hepatocytes incubated with [^{3}H]-2,3,7,8-TCDD at concentrations of 0.01, 0.1, and 1.0 µM (Olson et al., 1994). Lower 2,3,7,8-TCDD concentrations in the media result in concentrations in hepatocytes that are more similar to the levels in the liver after in vivo exposure. For example, the concentration of 2,3,7,8-TCDD in hepatocytes incubated at 0.01 µM is similar to hepatic levels after in vivo exposure of rats at a dose of ~10 µg/kg. At 0.01 and 0.1 µM, the rate of metabolism of [^{3}H]-2,3,7,8-TCDD was similar in hepatocytes isolated from control and 2,3,7,8-TCDD-pretreated rats, whereas at 1.0 µM, [^{3}H]-2,3,7,8-TCDD metabolism was greater in hepatocytes isolated from 2,3,7,8-TCDD-pretreated rats. The results indicate that 2,3,7,8-TCDD can induce its own rate of metabolism in the rat, but only at high hepatic concentrations, which are generally not attained after in vivo exposure. Therefore, in vitro studies of the hepatic metabolism of TCDD (at 0.01 and 0.1 µM) are consistent with the lack of autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion observed in vivo in the rat (Kedderis et al., 1991b; Curtis et al., 1990).

The metabolism of [^{3}H]-2,3,7,8-TCDF was also investigated in isolated rat hepatocytes incubated at concentrations of 0.01, 0.1, and 1.0 µM (Olson et al., 1994). At all concentrations, hepatocytes from 2,3,7,8-TCDD-pretreated rats metabolized 2,3,7,8-TCDF at a rate from 4- to 25-fold greater than that observed in hepatocytes from control rats. The results indicate that 2,3,7,8-TCDF is metabolized in rat liver by a 2,3,7,8-TCDD-inducible enzyme, possibly CYP1A1 or 1A2. These in vitro results support the in vivo autoinduction of 2,3,7,8-TCDF metabolism and biliary elimination observed in the rat (McKinley et al., 1993).

2,3,7,8-TCDF metabolism was also investigated in rat liver, kidney, and lung microsomes in the presence and absence of selective chemical inhibitors and antibodies to CYP1A1 and CYP1A2 (Tai et al., 1993). Together, the results of this investigation indicate that CYP1A1 is the primary enzyme responsible for the metabolism of 2,3,7,8-TCDF. 2,3,7,8-TCDF was also metabolized by recombinant yeast microsomes expressing human CYP1A1 and reductase. However, based on EROD activity, a marker of CYP1A1, the relative rate of 2,3,7,8-TCDF metabolism was about 100-fold greater in TCDD-induced rat liver microsomes than in yeast microsomes expressing human CYP1A1 and reductase (Tai et al., 1993). Although 2,3,7,8-TCDF was metabolized by rat and human CYP1A1, the results indicated that there are marked quantitative differences in metabolism that suggest that 2,3,7,8-TCDF will be more persistent in humans.

In summary, there are in vivo and in vitro data suggesting that autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism does not occur in the rat after exposure to sublethal doses

of these agents. This is in contrast to 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, where in vivo and in vitro results support the autoinduction of metabolism and biliary elimination of these compounds in the rat.

1.3.4. Excretion in Animals

Data regarding the excretion of 2,3,4,7-TCDD and related compounds after exposure to a single radiolabeled congener (see Table 1-9) support the assumption of a first-order elimination process consisting of one or more components. These studies show that 2,3,7,8-TCDD was excreted slowly from all species tested, with half-lives ranging from 11 days in the hamster to 2,120 days in humans. 2,3,7,8-TCDD is exceptionally persistent in humans relative to other animal models. Elimination data in tissues (see Tables 1-6 and 1-7) also indicate that 2,3,7,8-TCDD and related compounds are exceptionally persistent in nonhuman primates (Bowman et al., 1989; Neubert et al., 1990). These differences may also be in part related to the dose dependency of the excretion of these compounds. In general, the congener- and species-specific rates of elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (see Table 1-6) are similar to the excretion data summarized in Table 1-9.

In the Syrian Golden hamster, the mammalian species least sensitive to the acute toxicity of 2,3,7,8-TCDD, excretion occurred readily through both the urine (35% of administered dose, 41% of total excreted radioactivity) and feces (50% of the administered dose, 59% of total excreted radioactivity) (Olson et al., 1980). A similar excretion pattern, with significant urinary elimination, was observed in mice, although there was significant strain variability (Gasiewicz et al., 1983b; Birnbaum, 1986). In all the other species, excretion occurred mainly through the feces, with relatively minor amounts of 2,3,7,8-TCDD metabolites found in the urine (Piper et al., 1973; Allen et al., 1975; Olson, 1986; Rose et al., 1976; Gasiewicz and Neal, 1979; Pohjanvirta et al., 1990). Results in Table 1-9 also indicate that fecal elimination was the primary route for the excretion of 1,2,3,7,8-PeCDD, OCDD, 2,3,7,8-TBDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, and 3,3′,4,4′-TCB. Only Piper et al. (1973) reported the excretion of metabolites in the expired air. During 21 days following administration of a single oral dose of [^{14}C]-2,3,7,8-TCDD to rats, 3.2% of the administered radioactivity (4.6% of the excreted radioactivity) was recovered in the expired air.

Studies in the rat, guinea pig, hamster, and mouse have found that essentially all of the 2,3,7,8-TCDD-derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD (see Table 1-9). The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that, once formed, the metabolites of 2,3,7,8-TCDD are excreted readily. Thus, urinary and biliary elimination of 2,3,7,8-TCDD depends on metabolism of the toxin. The more limited data for other compounds also suggest that this relationship may be true for 1,2,3,7,8-

PeCDD, 2,3,7,8-TBDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, and 3,3′,4,4′-TCB (see Table 1-9).

Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data indicate that 2,3,7,8-TCDD and its metabolites are excreted in the feces of guinea pigs, rats, mice, and hamsters treated with [^{3}H]- or [^{14}C]-2,3,7,8-TCDD (see Table 1-9). Whereas 15% to 35% of the 2,3,7,8-TCDD-derived radioactivity in rat, mouse, and hamster feces represents unchanged 2,3,7,8-TCDD, 81% of the radioactivity in guinea pig feces represents unmetabolized 2,3,7,8-TCDD (Olson, 1986; Neal et al., 1982; Gasiewicz et al., 1983b; Olson et al., 1980). The daily presence of unchanged 2,3,7,8-TCDD in feces and its absence in bile suggest that direct intestinal elimination may be the source for the fecal excretion of 2,3,7,8-TCDD. Data also suggest that direct intestinal elimination of parent compound contributes to the fecal excretion for 2,3,7,8-TBDD (Kedderis et al., 1991a). Direct intestinal elimination of the parent compound may occur for other congeners (see Table 1-9), but this conclusion cannot be made at this time because of the lack of experimental data. Nonetheless, the species-specific fecal excretion of 2,3,7,8-TCDF is very similar to that observed for 2,3,7,8-TCDD, with >90% of the 2,3,7,8-TCDF-derived radioactivity excreted in guinea pig feces representing parent compound (Decad et al., 1981a). In addition, the excretion of unchanged CDDs and CDFs was detected in rat feces after subcutaneous exposure to a defined mixture of congeners (Abraham et al., 1989d). Studies in lactating rats have also found that unchanged 2,3,7,8-TCDD may be excreted in the milk of lactating animals (Moore et al., 1976; Lucier et al., 1975; Nau et al., 1986). Lactation, direct intestinal elimination, and perhaps sebum may serve as routes for excretion of 2,3,7,8-TCDD that do not depend on metabolism of the toxin. These data suggest that the in vivo half-life for elimination of 2,3,7,8-TCDD and related compounds provides only an approximation of the rate of metabolism of these compounds in a given animal. The results in Table 1-9 suggest that 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 3,3′,4,4′-TCB are metabolized and excreted more rapidly than 2,3,7,8-TCDD, 2,3,7,8-TBDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and OCDD.

The rate of excretion of 2,3,7,8-TCDD and related compounds is species- and congener-specific (see Table 1-9). 2,3,7,8-TCDD is most persistent in human and nonhuman primates. In the hamster, the least sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean $t_{½}$ was 10.8 days (Olson et al., 1980), and in the guinea pig, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean $t_{½}$ was 94 days (Olson, 1986). 2,3,7,8-TCDF was also most

persistent in the guinea pig, with a $t_{½}$ of 20 to 40 days (Decad et al., 1981a; Ioannou et al., 1983). Furthermore, results indicate that the relatively limited ability of the guinea pig to metabolize 2,3,7,8-TCDD and -TCDF may contribute to the greater persistence and greater acute toxicity of these congeners in the guinea pig.

The tissue distribution, metabolism, and excretion of 2,3,7,8-TCDD were also investigated in Han/Wistar and Long-Evans rats, which were, respectively, more resistant (LD_{50}>3,000 µg/kg) versus more susceptible (LD_{50} ~10 µg/kg) to the acute toxicity of 2,3,7,8-TCDD (Pohjanvirta et al., 1990). The results suggest that the metabolism and disposition of 2,3,7,8-TCDD do not have a major role in explaining the strain differences in toxicity.

The intraspecies differences in the $t_{½}$ of 2,3,7,8-TCDD in three mouse strains may be due to the finding that the DBA/2J strain possesses about twofold greater adipose tissue stores than the C57BL/6J and B6D2F_1/J strains (Gasiewicz et al., 1983b). The sequestering of the lipophilic toxin in adipose tissue stores of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain. Birnbaum (1986) examined the effect of genetic background on the distribution and excretion of 2,3,7,8-TCDD in two sets of congenic mouse strains in which the congenic pairs differed only at the Ah locus. The Ah locus had no effect on the tissue distribution or excretion of 2,3,7,8-TCDD. Thus, the distribution and excretion of 2,3,7,8-TCDD were primarily governed by the total genetic background rather than the allele present at the Ah locus. These findings are consistent with the in vitro results of Shen and Olson (1987), who found that the hepatic uptake and metabolism of 2,3,7,8-TCDD did not correlate with genetic differences at the murine Ah locus. However, it is important to note that all of these are relatively high-dose studies, which may not allow for detection of Ah receptor-mediated effects on disposition.

Although the dose-related tissue distribution of 2,3,7,8-TCDD and related compounds has been described recently, very limited data are available on the dose-related excretion of these compounds. Rose et al. (1976) investigated the elimination of [^{14}C]-2,3,7,8-TCDD in rats given repeated oral doses of 0.01, 0.1, or 1.0 µg/kg/day Monday through Friday for 7 weeks or a single dose of 1.0 µg/kg. In the single-dose study, no ^{14}C was excreted in the urine or expired air; in the repeated-dose study, however, 3% to 18% of the cumulative dose was excreted in the urine by 7 weeks. This study indicated that steady-state concentrations will be reached in the bodies of rats in ~13 weeks. The rate constant defining the approach to steady-state concentrations was independent of the dose of 2,3,7,8-TCDD over the range studied. Relatively small changes in the excretion of 2,3,7,8-TBDD were also observed after exposures at 1 and 100 nmol/kg (Kedderis et al., 1991a). These results are consistent with the in vivo and in vitro evidence suggesting that autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism does not occur in the rat after exposure to sublethal doses of these compounds (Kedderis et al., 1991b; Curtis et al., 1990; Olson et al., 1994). In contrast to these compounds, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF can induce

their own rate of metabolism and biliary excretion (Brewster and Birnbaum, 1987; McKinley et al., 1993; Olson et al., 1994). Autoinduction of metabolism suggests that these compounds may exhibit dose-related excretion, with longer half-lives for elimination at lower doses, which are not associated with enzyme induction. Further data are needed to test this hypothesis.

1.3.5. Excretion in Humans

Poiger and Schlatter (1986) investigated the excretion of 2,3,7,8-TCDD in a 42-year-old man (92 kg) after ingestion of 105 ng (1.14 ng/kg) [^{3}H]-2,3,7,8-TCDD in 6 mL corn oil (see Tables 1-9 and 1-10). The half-life for elimination was estimated to be 2,120 days based on fecal excretion over a 125-day period following the single exposure. The concentration of ^{3}H-TCDD-derived radioactivity was also measured in adipose tissue in the same individual over a 6-year period following exposure. A more accurate estimate of 2,3,7,8-TCDD half-life of 9.7 years was calculated based on adipose tissue concentrations over a 6-year period (Schlatter, 1991). Table 1-10 summarizes additional half-life estimates for 2,3,7,8-TCDD and related compounds in humans, on the basis of serum and adipose tissue concentrations at two or more time points.

The Air Force is conducting a 20-year prospective study of veterans of Operation Ranch Hand, the unit responsible for the aerial spraying of herbicides, contaminated with 2,3,7,8-TCDD, in Vietnam from 1962 to 1971. A subset of the Ranch Hand cohort has had a series of up to four serum 2,3,7,8-TCDD analyses conducted to investigate the elimination of 2,3,7,8-TCDD in humans. Initially, the half-life of 2,3,7,8-TCDD in humans was estimated to be ~7 years on the basis of 2,3,7,8-TCDD levels in serum samples taken in 1982 and 1987 from 36 of the Ranch Hand personnel who had 2,3,7,8-TCDD levels >10 ppt in 1987 (Pirkle et al., 1989). Subsequently, Wolfe et al. (1994) investigated the half-life of 2,3,7,8-TCDD in an expanded cohort of 337 Air Force veterans of Operation Ranch Hand that also included the 36 subjects of the earlier half-life study by Pirkle et al. (1989). Based on paired 2,3,7,8-TCDD measurements from serum collected in 1982 and in 1987, the authors reported a mean predicted half-life of 11.6 years and a median observed half-life of 11.3 years with a nonparametric 95% confidence interval of 10.0 to 14.1 years. The authors also investigated how the 2,3,7,8-TCDD half-life varied with percent body fat (PBF), relative changes in PBF from 1982 to 1987, and age. They found that the 2,3,7,8-TCDD half-life increased significantly with a high PBF, suggesting that persons with more body fat tend to eliminate 2,3,7,8-TCDD more slowly. In contrast, increasing age was associated with a shorter half-life. The redistribution of fat stores from subcutaneous to abdominal areas with aging, resulting in greater mobilization of 2,3,7,8-TCDD, could in part explain the shorter half-life observed in older veterans. An increase in PBF from 1982 to 1987 was also associated with a decrease in half-life, which can be explained by a dilution of the existing body burden of 2,3,7,8-TCDD into the increasing adipose tissue mass.

More recently, Michalek et al. (1996) estimated the half-life of 2,3,7,8-TCDD in 213 veterans of Operation Ranch Hand on the basis of 2,3,7,8-TCDD serum analyses conducted in 1982, 1987, and 1992. Of the 278 subjects with complete data in all 3 years, 213 were included for analysis of half-life 3 on the basis of 2,3,7,8-TCDD levels greater than 22.3 ppt in 1982, >14.9 ppt in 1987, and >10 ppt in 1992. All TCDD levels were background-corrected by subtracting 4 ppt, and the logarithm of the background-corrected levels were modeled as a linear function of time to estimate decay rates using first-order kinetics. Using the Toeplitz assumption, the unadjusted estimated decay rate is 0.0797 per year (95% CI of 0.0727 to 0.0868), giving an unadjusted half-life estimate of 8.7 years (95% CI of 8.0 to 9.5 years). The adjusted half-life was found to increase significantly with an increase in PBF in 1982, but the half-life did not vary with age or relative changes in PBF. Most recently, Michalek and Tripathi (1999) estimated the half-life of 2,3,7,8-TCDD in 97 veterans of Operation Ranch Hand on the basis of 2,3,7,8-TCDD serum analyses conducted in 1982, 1987, 1992, and 1997. Of the 244 subjects with complete data at all four time points, only 97 were included for analysis of half-life based on the criteria of 2,3,7,8-TCDD levels greater than 39.5 ppt in 1982, >25.0 ppt in 1987, >15.8 ppt in 1992, and >10 ppt in 1997. With increasing time since the initial exposure to 2,3,7,8-TCDD, a greater proportion of the population was excluded from the analysis, as more subjects approached background body burdens of 2,3,7,8-TCDD. Using the methods of the previous report (Michalek et al., 1996), the unadjusted estimated elimination rate was 0.0915 per year (95% CI of 0.0844 to 0.0986), giving an unadjusted half-life estimate of 7.6 years (95% CI of 7.0 to 8.2 years). Because of the smaller sample size, the current elimination rate estimate, based on four measurements per subject, has less precision than the earlier estimate of Michalek et al. (1996), which was based on three measurements per subject. Once again, the elimination rate decreased slightly but significantly as PBF increased, supporting the hypothesis that individuals with more body fat tend to eliminate TCDD more slowly than those with less body fat. Michalek and Tripathi (1999) also reported no significant change in the elimination rate with age or with relative changes in PBF.

The half-life of 2,3,7,8-TCDD has also been investigated in two additional populations. Flesch-Janys et al. (1996) studied a group of 43 German herbicide plant workers that had initial 2,3,7,8-TCDD serum levels from 15.6 to 300 ppt. A median half-life estimate of 7.2 years was reported for this occupational cohort, which received an initial exposure to 2,3,7,8-TCDD similar to that of the Ranch Hand veterans. A similar half-life estimate of 8.2 years was reported in 27 victims of the accident in Seveso, Italy (Needham et al., 1994). This cohort had a greater initial exposure, resulting in serum levels of 130 to 3,830 ppt 2,3,7,8-TCDD. This study also included the early and later portions of the 2,3,7,8-TCDD decay curve, as the initial blood sampling began immediately following exposure and continued for 15.9 years. Thus, based on results from the

Ranch Hand, the German, and the Seveso studies, the estimated half-life of 2,3,7,8-TCDD in humans is from 7.2 to 8.7 years (Table 1-10).

Half-life estimates for other CDDs and CDFs have been estimated to range from 0.8 to 19.6 years (Table 1-10). Some of the half-life values in Table 1-10 are rough estimates based on a small number of individuals and analysis at as few as two time points. Phillips (1989) discusses this issue. Estimates also assume a simple, single-compartment, first-order elimination process.

In the largest and most comprehensive study, Flesch-Janys et al. (1996) investigated the elimination of 2,3,7,8-chlorine substituted CDDs and CDFs in a cohort of workers from a herbicide-producing plant in Germany (summarized in Table 1-10). The study group consisted of 45 males and 3 females with a mean duration of employment of 13.1 years. Mean time between end of employment and first blood sample was 5.4 years (median 2 years) and mean time between first and last blood sample was 5.6 yr (median 6.3 years). A total of 43 subjects with 2 serum samples and 5 subjects with 3 serum samples were included in the study. For each congener, only those subjects whose congener serum levels exceeded 95% of German background concentration were included in the analysis. The mean background concentration was also subtracted from every original measurement before analysis. For 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and OCDF, no half-life was estimated because no person in the study passed the above inclusion criteria. 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF are excreted in animals much more rapidly than other congeners (van den Berg et al., 1994), suggesting that these congeners may also be excreted more rapidly in humans. Conversely, 2,3,4,7,8-PeCDF is far more persistent in animal models than is 2,3,7,8-TCDD, which supports the estimated 19.6-year half-life of 2,3,4,7,8-PeCDF and 7.2-year half-life of 2,3,7,8-TCDD in humans. However, this estimate was based on only five subjects who met the criteria for inclusion in the study. With the exception of 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD, the median half-lives of the CDDs are generally similar. The estimates for these two congeners may be somewhat unstable because of variable individual rate constants for elimination and the fact that about 25% of the population showed no decrease in serum levels over the sampling period. Furthermore, the investigation found that increasing age and PBF were associated with increasing half-life for most congeners. Finally, it is important to note that the half-life data reflect only the elimination of CDDs and CDFs from blood lipid and may not reflect elimination from different storage sites for all congeners. In the case of 2,3,7,8-TCDD, it can be assumed that the half-life estimate reflects elimination from the main storage site, because about 90% of the body burden is sequestered in fat and the blood fat/adipose tissue concentration is about 1 (Patterson et al., 1987; Kahn et al., 1988; Schecter et al., 1990a; van den Berg et al., 1994). Data are more limited on the relative amount of other congeners stored in adipose tissue in humans, and limited and somewhat conflicting data suggest that the blood fat/adipose tissue concentration ratio may increase up to a factor of 2 for OCDD (Schecter et al., 1990a; Gurn et

al., 1995). Thus, some uncertainties remain regarding the extent that the observed decrease in serum levels of higher CDDs and CDFs reflects the elimination of these compounds from the body.

Ryan and Masuda (1991) reported on their continuing investigation into the elimination of CDFs in humans from the Yusho and Yu-Cheng rice oil poisonings. Yu-Cheng individuals had CDF blood levels on a lipid basis of 1-50 µg/kg, whereas Yusho patients had levels of 0.1-5 µg/kg. In the Yu-Cheng individuals, half-lives for three CDFs were 2 to 3 years, whereas elimination from Yusho individuals was more variable and slower, with half-lives >5 years (see Table 1-10) and, in several cases, no measurable elimination during the 7 years in which samples were available. The limited results suggest that clearance of these CDFs in the human is biphasic, with faster elimination at higher exposure. Schecter et al. (1990b) and Ryan and Masuda (1989) also reported longer half-life values for CDFs in humans at later time points after exposure, when concentrations are closer to the background levels of individuals with no unusual exposure.

While results from animal studies (summarized in Table 1-9) suggest that direct fecal excretion of unmetabolized CDDs and CDFs represents a significant mechanism for the elimination of these lipophilic compounds, human data have been limited until recently. The mass balance study of Schlummer et al. (1998) provided the experimental human data in support of the two-step model of CDD and CDF transfer in the gastrointestinal tract, where absorption and excretion are distinct processes occurring at the small and large intestine, respectively (see Section 1.1.1.2). Rohde et al. (1999) conducted a digestive tract mass balance study of six German men (age 41 to 73 years) with occupational exposure to CDDs and CDFs. Blood lipid levels of the subjects in 1996 ranged from 84 to 505 pg/g lipid for 2,3,7,8-TCDD and 270 to 640 pg/g lipid for TEQs, compared with background levels in unexposed individuals of 5.2 and 32 pg/g lipid, respectively. The daily quantity of nonmetabolized 2,3,7,8-chlorine-substituted CDDs and CDFs excreted in the feces exceeded the daily uptake from food, indicating significant clearance across the gastrointestinal tract. The concentration of these compounds in feces was also found to be highly correlated with that in blood, demonstrating that the fecal CDD and CDF content was directly related to the body burden of these compounds. No significant clearance (excretion via feces at least fourfold greater than uptake by food) was observed for congeners, including 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,4,7,8,9-HepCDF, or OCDF, which were not markedly elevated in the serum lipids. Together, these results support the relationship that fecal excretion is regulated by the lipid-based blood concentration of these compounds. Because of fecal clearance of nonmetabolized congeners, the half-lives in these subjects were estimated from the excretion rate and current body burden and ranged from 10 years for OCDD to 22 years for 2,3,7,8-TCDD to 33 years for 2,3,4,7,8-PeCDF. Congener-specific half-lives, similar to those reported by Flesch-Janys et al. (1996), were also calculated based on the decrease in serum lipid

level of congeners between 1990/92 and 1996. The fecal clearance of nonmetabolized CDDs and CDFs contributed on average between 37% (2,3,7,8-TCDD) and 90% (OCDD) to the total elimination. Thus, fecal clearance plays an important role in the overall elimination of most congeners, with the daily fecal excretion estimated to be equivalent to the amount of TEQ present in about 1.7 g of blood lipids (Rohde et al., 1999).

Because direct fecal excretion is a significant route for the excretion of nonmetabolized CDDs and CDFs (Schlummer et al., 1998; Rohde et al., 1999), two recent studies investigated whether Olestra, a nonabsorbable sucrose-polyester synthetic fat substitute, may enhance the elimination of these compounds in humans. Moser and McLachlan (1999) compared the fecal excretion of CDDs, CDFs, PCBs, and HCB with background exposures in three subjects who ate an Olestra-free diet and who ate a diet supplemented with 25 g/day of Olestra. The fecal excretion while on the Olestra diet was 1.5 to 11-fold higher, depending on the congener. If fecal excretion is estimated to contribute 40% of the overall elimination of 2,3,7,8-TCDD, and the Olestra diet enhanced fecal excretion 5.7-fold, the overall rate of elimination of 2,3,7,8-TCDD would be more than doubled on the Olestra diet. Geusau et al. (1999) investigated the effect of an Olestra-supplemented diet on the excretion of 2,3,7,8-TCDD in two patients with chloracne and very high serum 2,3,7,8-TCDD levels of 144,000 and 26,000 pg/g lipid. A diet supplemented with fat-free potato chips (33 to 66 g Olestra/day) enhanced the fecal excretion of 2,3,7,8-TCDD by up to eight- to ten-fold. Results suggest that the increase in fecal excretion of 2,3,7,8-TCDD was due mainly to an increase in the amount of fat (dietary fat plus Olestra) excreted via the feces. The resulting elimination half-lives of 2,3,7,8-TCDD due to fecal excretion were estimated to be 1.4 years in the more highly exposed patient and 1.9 years in the other individual. However, half-lives of 200 days and 230 days, respectively, were determined through analysis of serum and adipose tissue 2,3,7,8-TCDD levels over the 8-month observation period. The observed half-lives were far shorter than could be explained by an enhanced fecal or other elimination mechanism. In addition to the enhanced fecal excretion with Olestra, the authors speculate that the high levels of 2,3,7,8-TCDD may have also induced the metabolism of 2,3,7,8-TCDD in these subjects, resulting in the shorter observed half-lives.

In related studies, Morita et al. (1993, 1995, 1997, 1999) investigated the role of dietary fiber or Chlorella in the fecal excretion of CDDs and CDFs in rats. Chlorella is a unicellular green algae, sold as a health food or health supplement. Chlorella in the diet of rats also enhanced the fecal excretion of CDDs from 0.8- to 5.6-fold and CDFs from 0.9- to 11.1-fold above that of rats on a control diet (Morita et al., 1999). Rice bran fibers enhanced the fecal excretion of CDDs from 0.6- to 2.3-fold and CDFs from 0.5- to 10.4-fold above that of rats on a control diet (Morita et al., 1997). Dietary fiber, chlorophyll, and/or lipid in the Chlorella may be factors responsible for the enhanced fecal excretion of CDDs and CDFs observed in this study. Thus, fiber and/or

Chlorella may be other dietary factors capable of increasing the fecal excretion of CDDs and CDFs.

Because of the lipophilic nature of milk, lactation can provide a relatively efficient mechanism for decreasing the body burden of 2,3,7,8-TCDD and related compounds in females. As discussed by Graham et al. (1986), this elimination of 2,3,7,8-TCDD through mother's milk can result in high exposure levels in the infant. The relatively high bioavailability of CDDs and CDFs from mother's milk in nursing infants was discussed earlier in Section 1.1.1.2. Further discussion of lactation as a route for excretion of CDDs and CDFs in women and exposure in infants is given in Section 1.4.5.

1.4. PHARMACOKINETICS AND EXPOSURE

1.4.1. Introduction

Pharmacokinetic models, often simple models that represent the human body or a specific tissue in the human body as a storage compartment for a chemical, can be used to relate doses to internal tissue concentrations or to some other metric of toxic action. These models are represented by a differential equation that accounts for the mass balance about the compartment. In the simplest case, the change in mass of a chemical within the storage compartment is equal to the difference between the mass entering the compartment and the mass leaving the compartment over a specified time period.

Physiologically based pharmacokinetic (PBPK) models are special extensions of the simple pharmacokinetic model. These models utilize multiple compartments to represent the different tissues or physiological regions of the body. They incorporate known or estimated anatomical, physiological, and physicochemical parameters to describe quantitatively the disposition of a chemical between different tissues. PBPK models are useful to extrapolate between high- to low-dose kinetics within a species, to estimate equivalent doses by different routes of administration, and to extrapolate doses across species (Scheuplein et al., 1990). Given some knowledge of possible exposure patterns and scenarios, PBPK models can be used in an inverse fashion to estimate exposures on the basis of internal body doses. Some examples of how to use simple PK and PBPK models to estimate exposure to 2,3,7,8-TCDD and related compounds will be given here. In addition, this section will utilize model estimations to examine some special cases of exposure, in particular, exposure to lactating infants and the elderly.

1.4.2. Estimating Daily Intake of TCDD

Because TCDD is highly lipophilic, it has been shown that a majority of the TCDD in any body tissue is stored in the fat (van der Molen et al., 1996). As a first approximation, a one-

compartment pharmacokinetic model with first-order elimination may be used to compute the daily intake of TCDD based on steady-state concentrations in the fat. The mass balance model is:

$$V_F \frac{dC_F}{dt} = D - k_e A_F \qquad (1\text{-}1)$$

where:

V_F = volume of fat

CF = concentration of TCDD in fat

D = daily dose (mass/time)

A_F = mass of TCDD in fat

k_e = first order elimination constant ($time^{-1}$)

Concentration, C_F, is given by:

$$C_F = \frac{A_F}{V_F} \qquad (1\text{-}2)$$

Then, at steady state ($dC_F/dt = 0$), daily dose exactly balances elimination.

$$D = k_e A_F = k_e V_F C_F \qquad (1\text{-}3)$$

Note that k_e can be expressed in terms of half-life:

$$k_e = \frac{ln2}{t_{1/2}} \qquad (1\text{-}4)$$

Substituting Equation 1-4 into Equation 1-3, one obtains the following expression for daily dose in terms of fat concentration:

$$D = \frac{ln2}{t_{1/2}} V_F C_F \qquad (1\text{-}5)$$

It is important to recall the two assumptions implicit in the derivation of the above formula for daily uptake. First, steady-state conditions are assumed. Given that the half-life of some of these compounds is long (e.g., for 2,3,7,8-TCDD the half-life is 7 years), steady-state levels would only be approached if the level of exposure were constant for 15-30 years. Pinsky and Lorber (1998) compiled data from several studies that indicate that environmental concentrations of TCDD and related compounds have been decreasing over the past 20-30 years. They use a single-compartment model similar to the one presented above, but with a time-varying exposure profile rather than the constant input. The profile was determined statistically on the basis of previously recorded environmental trends. Using the prior exposure knowledge, Pinsky and Lorber found that the pharmacokinetic model was better able to predict body burdens that have been recorded over time than was the steady-state model. By manipulating the non-steady-state model and comparing results to the steady-state approximation, it can be shown for a given body burden measurement, the steady-state approximation would result in an overestimate of daily intake. Using some estimates of the decreasing exposure function presented by Pinsky and Lorber (1998), it appears that the overestimate of daily intake could be 20% or more with the steady-state model.

Another assumption of the simple model presented in this section is that the elimination kinetics are assumed to be constant over the entire life of the individual. Because 2,3,7,8-TCDD and related compounds are stored primarily in fat, sudden weight loss and lactation would result in alterations of the TCDD elimination rate. Again, it would be assumed that for calculation of daily intake due to background exposure, the body burden data from such individuals would be identified and calculations handled accordingly.

Figure 1-3 shows a sample calculation for 2,3,7,8-TCDD using Equation 1-5. A fat volume of 14 L was chosen, representing 20% of the body weight. Also, for the purposes of this example, 1 mL of tissue was assumed to be equivalent to 1 g. Table 1-11 shows the estimated daily intake of 2,3,7,8-TCDD at several conditions. The range of daily intakes calculated is in agreement with those reported elsewhere (Fürst et al., 1991).

Thomaseth and Salvan (1998) developed a minimal PBPK model for 2,3,7,8-TCDD in humans and utilized this model to estimate occupational exposures to 2,3,7,8-TCDD. The model was reduced to one compartment for ease of solution and was based on the following assumptions: (1) dynamic equilibrium of 2,3,7,8-TCDD concentration between different body lipid distribution volumes, (2) first-order elimination proportional to 2,3,7,8-TCDD liver content, and (3) daily intake proportional to body weight. The best parameter estimates based on Ranch Hand data were obtained with log-transformed data under a mixed-effects model, with liver elimination $k_f = 0.022$ days^{-1} (95% CI of 0.02-0.024), and background input = 0.125 pg/kg/day (95% CI of 0.071-0.179). The model accounts for changes in body mass index (BMI) over time, with higher BMI being related to a longer half-life for 2,3,7,8-TCDD. The model was then used to estimate occupational exposure of 253 U.S. chemical plant workers for whom one measure of serum 2,3,7,8-TCDD was available. The estimated exposure of the NIOSH cohort was 233 pg/kg/day (95% CI of 192-273). This model is much more rigorous than the simple steady-state approximation, and if a PK model is to be used to attempt to estimate intake, this model would be preferable. However, solving this model for daily intake is more complex and requires some assumption of the pattern of exposure over time. Alternatively, one could estimate daily intake by direct exposure calculations, that is, by examining the interaction of humans with environmental media containing the highest concentrations of dioxins. More information on this is provided in the chapter on exposure (Chapter 4).

1.4.3. Daily Intake of Congeners

Steady-state average dose calculations may be performed for other congeners in a manner similar to that presented for 2,3,7,8-TCDD. Three pieces of information are necessary. First, concentrations in the adipose tissues must be known. Second, the half-lives of the compounds within the body must be known. Third, some understanding of the kinetics and exposure

conditions is required to ensure that steady-state conditions were achieved at the time of monitoring.

Concentrations of various congeners in adipose tissues can be found in several sources (Stanley et al., 1986; Schecter, 1991). Values range from around 2 ppt for 2,3,7,8-TCDF to several hundred ppt for 1,2,3,4,6,7,8,9-OCDD.

Half-lives could be determined from elimination data, if available. Methods have been suggested to determine the half-life of such compounds from uptake data relative to 2,3,7,8-TCDD. Schlatter (1991) has proposed one such method. The following has been adapted from that proposed method. Manipulation of Equation 1-5 results in:

$$C_{TCDD} = \frac{D_{TCDD} \quad t_{1/2,TCDD}}{V \ln 2} \tag{1-6}$$

For some other congener x:

$$C_x = \frac{D_x \quad t_{1/2,x}}{V \ln 2} \tag{1-7}$$

Thus, the ratio of concentrations of TCDD to x can be described by:

$$\frac{C_{TCDD}}{C_x} = \left(\frac{D_{TCDD} \quad t_{1/2,TCDD}}{V \ln 2} \right) \quad \left(\frac{V \ln 2}{D_x \quad t_{1/2,x}} \right) \tag{1-8}$$

Which, with algebraic manipulation and simplification, becomes:

$$t_{1/2,x} = \left(\frac{D_{TCDD} \quad t_{1/2,TCDD}}{C_{TCDD}} \right) \quad \left(\frac{C_x}{D_x} \right) \tag{1-9}$$

Assuming intake D to be mostly from food, especially animal-fat products, it can be related to absorption from these foods according to:

$$D_{TCDD} = (k_{a,TCDD}) \ (A_{TCDD}) \tag{1-10}$$

where:

$k_{a,TCDD}$ = absorption rate constant for TCDD

A_{TCDD} = concentration of TCDD in animal fat (diet)

and

$$D_x = (k_{a,x})\ (A_x) \qquad (1\text{-}11)$$

where:

$k_{a,x}$ = absorption rate constant for x

A_x = concentration of x in animal fat (diet)

As a result, the half-life for compound x can be described by:

$$t_{1/2,x} = t_{1/2,TCDD}\left(\frac{A_{TCDD}}{C_{TCDD}}\right)\left(\frac{C_x}{A_x}\right)\left(\frac{k_{a,TCDD}}{k_{a,x}}\right) \qquad (1\text{-}12)$$

When the absorption rate constants for each are equal or when the difference between them is small compared to differences in other parameters (concentration, half lives), Equation 1-12 can be further simplified to:

$$t_{1/2,x} = t_{1/2,TCDD}\left(\frac{A_{TCDD}}{C_{TCDD}}\right)\left(\frac{C_x}{A_x}\right) \qquad (1\text{-}13)$$

It should be noted that for some of these substances exposure is expected from other than food sources. For such cases Equation 1-12 would be modified to include these other sources as follows.

$$t_{1/2,x} = t_{1/2,TCDD}\left(\sum \frac{k_{a,i,TCDD}\ A_{i,TCDD}}{C_{TCDD}}\right)\left(\sum \frac{C_x}{k_{a,i,x}}\ A_{i,x}\right) \qquad (1\text{-}14)$$

where:

$k_{a,i,TCDD}$ = absorption rate constants for TCDD from each of the i media

$A_{i,TCDD}$ = concentration of TCDD in each of the i media

$k_{a,i,x}$ = absorption rate constants for x from each of the i media

$A_{i,x}$ = concentration of x in each of the i media

Other symbols: as previously defined

Again, if the differences between the absorption rate constants for TCDD and x are judged to be small, then the following variation of Equation 1-14 can be used:

$$t_{1/2,x} = t_{1/2,TCDD}\left(\sum\left(\left(\frac{A_{i,TCDD}}{C_{TCDD}}\right)\left(\frac{C_x}{A_{i,x}}\right)\right)\right) \quad (1\text{-}15)$$

Table 1-12 shows the results of some half lives calculated in this manner.

The half lives calculated using Equation 1-15 for the first three compounds in Table 1-12 agree with those calculated by Schlatter (1991). The large difference in the two calculations for OCDD is due to significant differences in absorption rates between TCDD and OCDD. Schlatter notes in his paper that for some compounds, including OCDD, corrections were made of differences in absorption. No explanation was offered on how this was done. However, Flesch-Janys et al. (1996) report a half-life of 6.7 years for OCDD. In addition, Flesch-Janys et al. (1996) report longer half-lives for 1,2,3,7,8-PeCDD (15.7 years) and 2,3,4,7,8-PeCDF (19.6 years). These differences could be due to a variety of factors including the dietary concentrations, absorption rates, other background sources, etc. Table 1-13 summarizes the results reported by Flesch-Janys.

When using this method to estimate daily intake of 2,3,7,8-TCDD and congeners, it should be noted that studies (Kim and Dubin, 1996; Shirai and Kissel, 1996) have indicated that there is considerable uncertainty associated with half-life measurements published in the literature.

1.4.4. Induction of Liver Binding Proteins and Resultant Distribution

Andersen et al. (1997a) developed a multicompartment geometric model of the liver in relation to regional induction of cytochrome P-450s. The model was based on five morphological and functional zones of the liver acinus: a concentric periportal zone, a fenestrated periportal region that interconnects among multiple functional units, and three concentric centrilobular areas. At low doses, 2,3,7,8-TCDD exhibits centrilobular expression of CYP1A1, and with increasing dose, adjacent areas radiating out from the centrilobular region also begin to express CYP1A1. To create realistic total induction curves that are relatively smooth, the differences in K_d (dissociation constant) values between adjacent subcompartments must be less than fivefold. Because of the high n (Hill constant) values, the low-dose induction characteristics predicted with the multicompartment liver model differ significantly from those predicted with a model that considers the liver as a single homogeneous compartment. A physiologically based pharmacokinetic (PBPK) model for 2,3,7,8-TCDD was then combined with a five-compartment geometric model of hepatic zonation to predict both total and regional induction of CYPs within

the liver (Andersen et al., 1997b). The model predicts an 81-fold difference in the affinity of the AhR-TCDD complex binding to DNA response elements for CYP1A1 between the centrilobular and the periportal regions. The PBPK analysis based on the multicompartment liver model suggests that the low-dose behavior for hepatic CYP1A1/1A2 induction by 2,3,7,8-TCDD is highly nonlinear.

1.4.5. Pregnancy and Lactation (Exposure of Offspring)

The distribution and excretion of [^{14}C]-2,3,7,8-TCDD (30 µg/kg) and [^{14}C]-2,3,7,8-TCDF (800 µg/kg) were studied in pregnant C57BL/6N mice after oral exposure on gestation day 11 (Weber and Birnbaum, 1985). The distribution and excretion of 2,3,7,8-TCDD and 2,3,7,8-TCDF in pregnant mice were similar to those of males of the same strain (Gasiewicz et al., 1983b; Decad et al., 1981b) (see Tables 1-6 and 1-9), although elimination rates were higher in the pregnant mice for both congeners. For 2,3,7,8-TCDD, liver, urinary, and fecal elimination was 3.0, 3.4, and 14.4 times faster than that reported for males. For 2,3,7,8-TCDF, liver, urinary, and fecal elimination was 1.3, 1.8, and 1.8 times faster than that observed for males. Elimination data from pregnant mice were based on only three time points (gestation days 12, 13, and 14) and thus represent only rough estimates. In addition, the greater fecal excretion could have been due to incomplete absorption of 2,3,7,8-TCDD after oral exposure. Although these results need further substantiation, it is conceivable that the sex of the animal, pregnancy, and the route of exposure could have a significant impact on the pharmacokinetics of these compounds.

In a related study, Krowke (1986) compared the 2,3,7,8-TCDD concentrations in the livers of pregnant and nonpregnant NMRI mice exposed subcutaneously to 12.5 or 25 nmol/kg/day on gestation days 9-11. At 7 days after exposure to the lower dose, the hepatic 2,3,7,8-TCDD concentrations were 7 and 32 ng/g in pregnant and nonpregnant mice, respectively. At the higher exposure, 5.5 times lower concentrations of 2,3,7,8-TCDD were found in the livers of pregnant animals on gestation day 18. A similar effect on hepatic 2,3,7,8-TCDD levels was observed in combined exposure, which contained 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, or 2,3,7,8-TCDF. The decreased hepatic levels of 2,3,7,8-TCDD in pregnant mice are consistent with the Weber and Birnbaum (1985) observation of more rapid elimination of 2,3,7,8-TCDD in pregnant mice. Further investigations are necessary to better characterize the apparently significant effects of pregnancy on the disposition of 2,3,7,8-TCDD and related compounds.

Weber and Birnbaum (1985) also investigated the distribution of [^{14}C]-2,3,7,8-TCDD (30 µg/kg) and [^{14}C]-2,3,7,8-TCDF (800 µg/kg) to the embryos of pregnant C57BL/6N mice after oral exposure on gestation day 11. On gestation days 12, 13, and 14, the percent of the maternal dose in the embryo remained constant at 0.032%-0.037%/embryo, while the concentrations in the

embryo were 0.34%, 0.17%, and 0.15% of the dose/g embryo, respectively. Embryos had approximately 11-fold higher concentrations of 2,3,7,8-TCDD than 2,3,7,8-TCDF when exposed on a percent of total dose/g tissue basis. This may be due to the more rapid metabolism and excretion of 2,3,7,8-TCDF compared with 2,3,7,8-TCDD. Assuming that all radioactive material found in embryos was parent compound, at most 2.6 ng (8 pmol) of 2,3,7,8-TCDD and 6.4 ng (21 pmol) of 2,3,7,8-TCDF/g tissue were detected under these conditions.

The transfer of [^{14}C]-2,3,7,8-TCDD to the embryo during early gestation was assessed in NMRI mice given a dose of 25 µg/kg by intraperitoneal injection on days 7, 8, 9, 10, 11, or 13 of gestation (Nau and Bass, 1981). The mice were sacrificed after 48 hours, and 2,3,7,8-TCDD concentrations were determined by liquid scintillation counting of solubilized tissue and by GC-ECD and GC/MS. Similar results were given by these methods, suggesting that 2,3,7,8-TCDD-derived [^{14}C] in maternal and embryonic tissue was the parent compound. The maternal liver contained from 4% to 8% of the dose/g, or 40-80 ng/g. 2,3,7,8-TCDD in embryonic tissue from gestation days 11-15 ranged from 0.04% to 0.1% of the dose/g, or 0.4-1.0 ng/g. In contrast, higher levels were found earlier in gestation, with 10 ng/g embryo on gestation day 9 and 2 ng/g on day 10. The higher levels may be related to placentation, which occurs at approximately gestation days 10–11 in this mouse strain. The affinity of fetal liver for 2,3,7,8-TCDD was relatively low, as compared to maternal liver; however, 2,3,7,8-TCDD levels in fetal livers were 2 to 4 times higher than levels in other fetal organs. Nau and Bass (1981) also attempted to correlate 2,3,7,8-TCDD levels in the fetuses with the observed incidence of cleft palate. Three groups of mice were given either a single intraperitoneal exposure to 25 µg/kg 2,3,7,8-TCDD on gestation day 7 or 10 or 5 µg/kg/day, intraperitoneally, on gestation days 7-11. On gestation day 13, 2,3,7,8-TCDD concentrations in maternal tissues were very similar in the three exposure groups. At day 13, however, the embryo contained 0.038±0.011% (0.36 ng/g), 0.096±0.027% (0.92 ng/g), and 0.12±0.05% (1.1 ng/g) of the dose (mean±SD) in the 7-, 10-, and 7- to 11-day exposure groups, respectively. Cleft palate incidence on gestation day 18 was 16%, 84%, and 65% for the 7-, 10-, and 7- to 11-day exposure groups, respectively. Although further studies are needed, these results suggest that cleft palate incidence is generally related to the 2,3,7,8-TCDD concentration in the embryo. In a related study, Couture et al. (1990) found that gestation day 12 was the peak period of sensitivity for 2,3,7,8-TCDD-induced cleft palate in C57BL/6N mice; however, tissue levels were not investigated.

In the same laboratory, Abbott et al. (1989) investigated the distribution of 2,3,7,8-TCDD in the C57BL/6N mouse fetus following maternal exposure on gestation day 11 to 30 µg/kg. 2,3,7,7-TCDD was detected in the gestation day-11 embryo at 3 hours postexposure and was equally distributed between the embryonic head and body. At 72 hours postexposure, 0.035% of the total dose was in fetal tissues and 1% of the 2,3,7,8-TCDD in the fetus (1.4-3.5 pg) was

found in the palatal shelf. More recently, Abbott et al. (1996) found that 2,3,7,8-TCDD was detected in maternal blood, liver, and fat and in the placenta, embryonic liver, and palate within 30 min after oral exposure of mice on gestation day 12. The levels peaked in blood and placenta at 3 hours and in the other tissues at 8 hours. At 24 hours following a single oral dose of 24 µg TCDD /kg, the above respective tissues contained 0.25, 98.8, 72.9, 1.22, 1.03, and 0.44 ng TCDD/g.

Krowke (1986) also measured the concentration of 2,3,7,8-TCDD in the placenta, amniotic fluid, and fetus of NMRI mice exposed to 2.5 nmol/kg by subcutaneous injection on days 9-11 of gestation. Similar concentrations of 2,3,7,8-TCDD were observed in the placenta, amniotic fluid, and fetus (~0.5 ng/g) on day 16 of gestation. Fetal liver 2,3,7,8-TCDD concentrations were at least five times greater than in other fetal tissue. Krowke (1986) reported slightly lower 2,3,7,8-TCDD levels in the fetal head relative to other extrahepatic fetal tissue, while Weber and Birnbaum (1985) found a slightly higher 2,3,7,8-TCDD concentration in the head relative to other extrahepatic fetal tissue.

Nau et al. (1986) investigated the transfer of 2,3,7,8-TCDD via the placenta and milk in NMRI mice exposed to 25 µg/kg on day 16 of gestation. The authors confirmed the relatively low fetal tissue levels with prenatal exposure to 2,3,7,8-TCDD (Nau and Bass, 1981) and found that postnatally 2,3,7,8-TCDD was transferred efficiently to mouse neonates and offspring by lactating mothers. During the first 2 postnatal weeks, the pups were given doses of 2,3,7,8-TCDD via the milk that were, on a body-weight basis, similar to those that had been administered prenatally to their mothers. 2,3,7,8-TCDD levels in the tissue of lactating mothers decreased within the first 3 postnatal weeks by two to three orders of magnitude to reach levels that were only ~2% of the corresponding levels in the pups that these mothers had nursed. Thus in mice, excretion into milk represents a major pathway for maternal elimination of 2,3,7,8-TCDD and for the subsequent exposure of pups.

The disposition of 2,3,7,8-TCDD in rat pups was assessed after the prenatal (via placental transfer) and postnatal (via milk) exposure from pregnant Wistar rats given a single dose of 3, 30, or 300 ng/kg, subcutaneously, on day 19 of gestation (Korte et al., 1990). Lactation resulted in the rapid elimination of 2,3,7,8-TCDD from maternal tissues, with the half-life of 2,3,7,8-TCDD in the liver of lactating rats estimated to be ~7 days. This compares to a half-life of 13.6 days in the liver of nonlactating rats (Abraham et al., 1988). At postnatal day 7, exposure via the milk resulted in pup liver 2,3,7,8-TCDD concentrations that were greater than the corresponding levels in maternal liver. In cross-fostering experiments, the concentrations of 2,3,7,8-TCDD in the liver of offspring at postnatal day 7 were 0.47, 2.59, and 4.16 ng/g in the 300 ng/kg groups exposed through the placenta only, via the milk only, and through the placenta and via the milk,

respectively. These results support the earlier observations that the placental transfer of 2,3,7,8-TCDD in rats and mice is relatively limited compared with the efficient transfer via maternal milk.

van den Berg et al. (1987b) investigated the transfer of CDDs and CDFs to fetal and neonatal rats. Prenatal exposure of the fetus was assessed in pregnant Wistar rats fed a diet containing a fly ash extract from a municipal incinerator on days 10-17 of gestation. Postnatal exposure of 10-day-old pups was assessed through feeding lactating mothers the same contaminated diet for the first 10 days after delivery. Although the fly ash extract contained almost all of the 136 tetra– to octa-CDDs and -CDFs, only 17 CDD and CDF congeners were detected as major compounds in the tissue of fetuses, pups, and dams. All of the congeners were 2,3,7,8-substituted with the exception of 2,3,4,6,7-PeCDF. 2,3,7,8-TCDD had the highest retention (0.0092% of the dose/g) in the fetus, while 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and hepta- and octa-CDDs and -CDFs were not detected in the fetus. In the liver of offspring, the highest retention was found for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and the three 2,3,7,8-substituted HxCDDs (0.74%-1.13% dose/g). The 2,3,7,8-substituted penta- and hexachlorinated congeners showed the highest retention in the livers of dams (2.05%-5.17% of dose/g liver), while 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,6,7-PeCDF had the lowest retention. A linear relationship was found between the retention of CDDs and CDFs in the livers of pregnant and lactating rats. Furthermore, a linear relationship was found between the retention of CDDs and CDFs in the livers of the lactating rats and livers of the offspring.

In a related study, Hagenmaier et al. (1990) investigated the transfer of CDDs and CDFs through the placenta and via milk in a marmoset monkey. A defined mixture of CDDs and CDFs was given as a single subcutaneous injection to a pregnant marmoset monkey at the end of the organogenesis period (week 10 of gestation, 11 weeks prior to delivery). Transfer of CDDs and CDFs through the placenta was investigated in a newborn 1 day after birth, and transfer through the placenta and via milk was assessed in an infant of the same litter after a lactation period of 33 days. Tissue concentrations of the offspring were compared with those of the mother at the end of the lactation period and with data from other adult marmosets obtained at this time of maximum absorption (1 week after injection) and 6 weeks after injection. Deposition of CDDs and CDFs into the newborn liver was very low, suggesting very little transplacental transport and hepatic accumulation of these compounds. 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD were found at the highest concentration in the liver of the newborn (~0.15% of dose/g). For all other congeners, the concentrations in the liver of the newborn were <10% of the corresponding concentrations in adults. In contrast to liver, concentrations of 2,3,7,8-substituted congeners in the adipose tissue of the newborn were at least 33% of the levels in adults, and in the case of OCDD and OCDF, levels were threefold higher in the newborn than in the adult. The adipose tissue/liver concentration ratios for 2,3,7,8-substituted congeners in the newborn ranged from 2.2 for

1,2,3,4,6,7,8-HpCDF to 10.9 for 2,3,7,8-TCDF. Furthermore, the concentration of these congeners in the newborn was highest in the adipose tissue, followed by the skin and liver. This is in contrast to the relative distribution in the adult, where the liver generally contains the highest levels of these congeners. The results indicate that hepatic concentrations in the fetus may not be representative of the rate of placental transfer of CDDs and CDFs. In the marmoset monkey, substantial placental transfer into fetal adipose tissue can be observed for most of the 2,3,7,8-substituted congeners during the fetal period. As expected from rodent studies, the transfer of CDDs and CDFs via mothers' milk was considerable, resulting in hepatic concentrations of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,6,7,8-HxCDD in the suckled infant (postnatal day 33) higher than those in the dam. The hepatic concentration of 2,3,7,8-TCDD in the 33-day-old infant was ~0.9% of the dose/g tissue. Transfer of hepta- and octa-CDDs and CDFs to the suckled infant was rather low, only ~10% of the levels in the dam. When total exposure of the mother and offspring at the end of the 33-day nursing period was assessed in terms of I–TE factors (U.S. EPA, 1989), the liver of the mother contained 2,494 pg I–TE/g, whereas the offspring liver contained 2,022 pg I-TE/g. This approach is necessary to assess total exposure due to the congener-specific transfer via lactation.

The pre– and postnatal transfer of 2,3,7,8-TCDD to the offspring of rhesus monkeys was investigated by Bowman et al. (1989). Animals were fed a diet containing 2,3,7,8-TCDD at concentrations of 5 or 25 ppt for ~4 years and were on a 2,3,7,8-TCDD-free diet for ~18 months prior to parturition. Maternal 2,3,7,8-TCDD levels (mean±SE) in adipose tissue were 49±11 (n=7) and 173±81 (n=3) ppt in the 5 and 25 ppt groups, respectively. Corresponding levels in the adipose tissue of offspring at weaning (4 months) were 187±58 and 847±298 ppt in the 5 and 25 ppt groups, respectively. From these data, a 2,3,7,8-TCDD BCF of 4.29 was estimated from mother to nursing infant. This value is similar to that observed for 2,3,7,8-TCDD in the marmoset monkey (Hagenmaier et al., 1990). The milk of the rhesus monkeys in the 25 ppt group contained from 4 to 14 ppt of 2,3,7,8-TCDD, which corresponds to 150-500 ppt on a lipid basis. The authors calculated that the three mothers in the 25 ppt group excreted from 17% to 44% of their 2,3,7,8-TCDD body burden by lactation. They also concluded that the results are generally consistent with overall triglyceride movement as mediating the excretion of 2,3,7,8-TCDD in milk.

In a subsequent study, Bowman et al. (1990) reported the relative persistence of 2,3,7,8-TCDD in the offspring of rhesus monkeys that were exposed earlier to 5 or 25 ppt of 2,3,7,8-TCDD in the diet. The concentration of 2,3,7,8-TCDD in adipose tissue was measured in offspring at ~4-5, 12, and 24 months of age. The decrease of 2,3,7,8-TCDD levels in adipose tissue of seven young monkeys departed somewhat from first-order, single-compartment kinetics, but with the limited data and an assumption of first-order kinetics, a half-life of 121 days was

estimated. When the data were adjusted within each animal for body weight gain and for average fat content at each age, the adjusted data apparently followed first-order, single-compartment kinetics, with a half-life of ~181 days. Thus, young monkeys apparently eliminate 2,3,7,8-TCDD from adipose tissue at a faster rate than adult rhesus monkeys, which had individual half-lives ranging from 180 to 550 days (Bowman et al., 1989).

Several studies provide data in support of the transplacental transport of CDDs and CDFs to the human fetus. Kreuzer et al. (1997) measured the concentration of CDDs and CDFs in the lipids of adipose tissue and livers of 3 stillborn infants and detected 16 of a possible 17 congeners, with the exception of 1,2,3,7,8,9-HxCDF. TEQ levels ranged from 6.2 to 10.8 pg/g lipid, and 2,3,7,8-TCDD levels ranged from 0.8 to 2.1 pg/g lipid for human infants that died at birth. These levels are similar to those reported in the lipid fractions of maternal tissues, suggesting that prenatal exposure to CDDs and CDFs reflects the levels present in maternal tissues. Similar findings were reported earlier by Schecter et al. (1990c), who detected 2,3,7,8-TCDD (1.3 to 4.3 pg/g lipid) in the livers of three stillborn infants. Thus, significant prenatal exposure to CDDs and CDFs occurs, with the concentration of these compounds in the lipids of the newborn infants generally reflecting that in maternal lipids.

A significant source of postnatal exposure of human infants to CDDs and CDFs is through the ingestion of human milk. Several investigators have quantified the levels of 2,3,7,8-TCDD in human milk samples. Many of the milk samples were pooled (Jensen, 1987). Rappe (1984) reported levels of 1-3 ppt 2,3,7,8-TCDD in milk fat (lipid adjusted) from five volunteers in West Germany, and in a later report, Rappe et al. (1985) reported an average level of 0.6 ppt 2,3,7,8-TCDD in milk fat from four volunteers in northern Sweden. Furst et al. (1986) reported an average level of 9.7 ppt 2,3,7,8-TCDD in milk fat from three individuals in the Netherlands and <1.0 ppt 2,3,7,8-TCDD in milk fat from two individuals in Yugoslavia. Nygren et al. (1986) reported average levels of 2,3,7,8-TCDD in human milk samples from four subjects in Sweden to be 0.6 ppt in milk fat, in five subjects from West Germany to be 1.9 ppt in milk fat, and in four subjects from Vietnam to be <0.5 ppt in milk fat.

High levels of 2,3,7,8-TCDD have been detected in the milk of mothers exposed to high levels of 2,3,7,8-TCDD in the environment. Reggiani (1980) reported levels between 2.3 and 28.0 ppt 2,3,7,8-TCDD in whole milk from mothers in Seveso. Baughman (1975) reported levels between 40.0 and 50.0 ppt 2,3,7,8-TCDD in whole milk from mothers in South Vietnam. Schecter et al. (1987) also found high ppt levels of 2,3,7,8-TCDD in human milk samples from South Vietnam. These authors found that levels from samples taken in 1985 from South Vietnamese mothers were comparable to the level of 2,3,7,8-TCDD currently found in North American human milk samples (5 ppt).

Furst et al. (1989) examined the levels of CDDs and CDFs in human milk and the dependence of these levels on the period of lactation. The mean concentrations of CDDs in human milk (on a fat basis) ranged from 195 ppt for OCDD to 2.9 ppt for 2,3,7,8-TCDD, with the levels of the other congeners decreasing with decreasing chlorination. This is in contrast to the generally lower levels of CDFs in human milk, which range from 25.1 ppt for 2,3,4,7,8-PeCDF to 0.7 ppt for 1,2,3,7,8-PeCDF. An evaluation of the CDD and CDF levels in relation to the number of breast-fed children found that the concentrations in milk generally decreased with the greater number of children. The CDD and CDF levels in milk from mothers nursing their second child are on average 20%-30% lower than those for mothers breast-feeding their first child. CDD and CDF levels were also analyzed in one mother over a period of 1 year after delivery of her second baby to assess the effect of duration of lactation. After breast-feeding for 1 year, the mother had CDD and CDF levels that were 30%-50% of the starting concentration. Levels in milkfat (ppt) at 1, 5, and 52 weeks after delivery were 251, 132, and 119 for OCDD; 7.9, 5.9, and 1.4 for 2,3,7,8-TCDD; and 33.1, 24.5, and 10 for 2,3,4,7,8-PeCDF, respectively. The results suggest a more rapid mobilization of CDDs and CDFs and excretion into human milk during the first few weeks postpartum. Although further studies are necessary, the limited data suggest that there are time-dependent, isomer-specific differences in the excretion of CDDs and CDFs in human milk.

Schecter et al. (1998a) assess the decrease in the levels of CDDs, CDFs, PCBs, DDE, and HCB in the blood and milk lipid in a mother that nursed twins over a 38-month period. During the first 23 months of nursing, the CDD and CDF TEQ decreased 68% (15.7 to 5.0 ppt, lipid) for blood and decreased 77% (13.6 to 3.1 ppt, lipid) for breast milk. Thus, lactation results in a similar reduction in CDD and CDF concentrations in the lipid fractions of blood and milk. During the first 23 months of nursing, the PCB 126 (3,3',4,4',5-PeCB) milk concentration also decreased 71% (21.0 to 6.1 ppt, lipid). The authors estimate that approximately 115 ng TEQ (CDDs, CDFs, coplanar PCBs) was ingested by each infant from breast feeding for this extended period of time.

Abraham et al. (1996) investigated the intake, fecal excretion, and blood levels of CDDs, CDFs, and PCB 126 in two breast-fed and two formula-fed infants. At 1 month, the concentrations of CDDs and CDFs in breast milk were 19.7 and 22.2 TEQ (pg/g lipid), while the formula diet contained only 0.38 TEQ (pg/g lipid). At the age of 11 months, the breast-fed infants' blood CDD and CDF concentrations were 29.2 and 37.5 TEQ (pg/g lipid), whereas the formula-fed infants' blood CDD and CDF concentrations were 2.4 and 2.6 TEQ (pg/g lipid). At this time, the mothers that breast-fed had blood CDD and CDF concentrations of 12.3 and 10.5 TEQ, while the mothers that formula-fed had blood levels of 16.9 and 13.8 TEQ (pg/g lipid). Because PCB 126 has a TEF of 0.1, it is important to note that at 11 months, breast-fed infants

also have much higher levels of PCB 126 (287 and 374 pg/g lipid) relative to formula-fed infants (24 and 18 pg/g lipid). PCB 126 levels in mothers that breast-fed were 105 and 86 relative to levels of 193 and 52 (pg/g lipid) in the mothers that formula-fed. Thus, when PCB 126 is included in the TEQ calculation, the breast-fed infants' total TEQ blood concentration (body burden) is more than doubled from that estimated on the basis of CDDs and CDFs alone. The results of this study provide direct, quantitative data showing that the body burden (blood level) of CDDs, CDFs, and PCB 126 is more than 10 times higher in 11-month-old breast-fed infants than in 11-month-old formula-fed infants.

Although data are more limited for the co-planar PCBs, 3,3′,4,4′-TCB, 3,3′,4,4′,5-PeCB, and 3,3′,4,4′,5,5′-HxCB have been detected in human milk from Swedish mothers, at concentrations of 16-32, 72-184, and 46-129 ppt on a fat basis, respectively (Noren et al., 1990). Therefore, lactation appears to be an effective means for the excretion of co-planar PCBs from mothers and a major source of postnatal exposure of nursing infants. Because 3,3′,4,4′,5-PeCB and other co-planar PCBs are present in human milk at concentrations up to 60-fold higher than 2,3,7,8-TCDD, it is important to consider the relative toxic potency of these dioxin-like compounds and their potential health impact on nursing infants.

Kreuzer et al. (1997) measured the levels of CDDs and CDFs in the lipids of adipose tissue and liver of 17 infants (0.43 to 44 weeks of age) who died of sudden infant death syndrome. As expected, the concentrations of these compounds in breast-fed infants were higher than those in non-breast-fed infants; however, the magnitude of this difference varied because of differences in the age of the subjects and the duration of breast-feeding. The TEQ concentrations in the livers of these subjects were slightly, but not significantly, higher than the respective levels measured in the adipose tissue lipids. The results also suggest that the higher chlorinated congeners preferentially accumulate in liver lipids, an observation made earlier for adults in a study by Thoma et al. (1990) (see Table 1-5).

Kreuzer et al. (1997) used data from the above study and other published results to validate a physiological toxicokinetic model they developed to describe the body burden of 2,3,7,8-TCDD for the entire human lifetime and the influence of breast-feeding on the body burden. The model includes gender- and age-dependent changes in the following parameters: body weight; volumes of liver, adipose, and muscle tissue; food consumption; and excretion of feces. The model also assumes that 2,3,7,8-TCDD exposure occurs primarily from the ingestion of contaminated food, that TCDD is distributed freely in lipids, and that TCDD is excreted unchanged in the lipids of the feces as well as following hepatic metabolism. More complex biochemical processes such as protein binding, saturation of metabolism at high 2,3,7,8-TCDD concentrations, or induction of metabolism are not part of this model, which considers factors more relevant for low-level or background human exposures. With the basis assumption of this

single-compartment model and the free distribution of 2,3,7,8-TCDD in all body lipids, including the gastrointestinal tract, the half-life of the nonmetabolic elimination (tf1/2) is proportional to the ratio of volume of body lipids (V) to the mass of lipids in stool excreted per unit time (dFa/dt). During aging, V increases at least 40 times but dFa/dt only 1.7 times (from 3 g/day in infants to 5 g/day in adults). Consequently, the half-life of the nonmetabolic elimination (tf1/2) is calculated to be only 0.42 years in newborns and 9.5 years in 40-year-old adults. According to this model, most 2,3,7,8-TCDD is eliminated as unchanged compound in children, with the role of metabolism-dependent elimination becoming more important with age. Thus, the half-life increases almost linearly from its starting value of about 4 months in newborns and reaches a value of 5 years at the age of 40. An age dependent elimination of 2,3,7,8-TCDD has also been reported experimentally in the rhesus monkey (Bowman et al., 1989, 1990). Furthermore, the model of Kreuzer et al. (1997) predicts that the relatively high 2,3,7,8-TCDD concentrations that might be reached after 6 months of nursing do not lead to an elevated lifetime body burden of 2,3,7,8-TCDD.

In a related study, Patandin et al. (1999) investigated dietary, including lactational, exposure to CDDs, CDFs, and PCBs from early childhood until the early reproductive age of 25 years in order to assess exposure risk to the next generation. Based on the analysis of 83 milk samples, previously reported analysis of food products, and food questionnaire data, the daily TEQ intake per kg body weight is 50 times higher in breast-fed than bottle-fed infants and 3 times higher in toddlers than in adults. Although exposures are relatively high in breast-fed infants, breast-feeding for 6 months contributes only 12% and 14% to the respective body burdens of men and women at the age of 25 years. After babies are weaned, dairy products, processed foods, and meat are major sources of exposure to these compounds.

1.4.6. Pharmacokinetics and Aging

The influence of aging on the intestinal absorption of 2,3,7,8-TCDD was studied in 13-week-, 13-month-, and 26-month-old (senescent) male Fischer 344 rats (Hebert and Birnbaum, 1987). Absorption was measured by an in situ intestinal recirculation perfusion procedure. When absorption was calculated in terms of ng 2,3,7,8-TCDD absorbed/g mucosal dry weight/hour, the decrease between the senescent rats and the two younger age groups, from 544 ng/g/hour (young) to 351 ng/g/hour (senescent), was not statistically significant ($p<0.05$). The results indicate that, as with other molecules that depend on diffusion for their absorption, aging does not affect the intestinal absorption of 2,3,7,8-TCDD.

Banks et al. (1990) studied the effect of age on the dermal absorption and disposition of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF in male Fischer 344 rats. When rats were administered the same dose per body weight, dermal absorption of 2,3,7,8-TCDD at 3 days after exposure

decreased from 17.7±2.7% (mean±SD) to 5.6±2.5% of the administered dose in 10- and 36-week-old rats, respectively. Dermal absorption in the 96-week-old rats was similar to that of the 36-week-old rats. Dermal absorption of 2,3,4,7,8-PeCDF also decreased from 22.2±0.2% to 14.7±3.8% of the administered dose in 10- and 36-week-old rats, respectively. Dermal absorption of both compounds was also decreased in older rats given the same total dose per surface area. Older animals may have decreased blood flow in the upper dermis, which will decrease the clearance of these compounds from the application site. Potential age-related changes in the intercellular stratum corneum lipids may also play a role in the decreased dermal absorption observed in older animals. Changes in the percentage of the administered dose detected in various depots reflected age-related changes in dermal absorption, whereas age-related changes in the tissue distribution of the absorbed dose reflected changes in the total mass of these tissues at various ages. Overall elimination of the absorbed dose was not affected by age. Although this investigation was conducted with a lipophilic solvent system and an animal model with skin that is more permeable than human skin, the results suggest that systemic bioavailability after dermal exposure to 2,3,7,8-TCDD or 2,3,4,7,8-PeCDF may be reduced in older age groups.

In a similar study, absorption, tissue distribution, and elimination were examined 72 hours after dermal application of a lower dose of 200 pmol (111 pmol/cm^2) 2,3,7,8-TCDD to weanling (3-week-old), juvenile (5-week-old), pubescent (8-week-old), young adult (10-week-old), and middle-aged (36-week-old) rats (Anderson et al., 1993). Dermal absorption using 2acetone as vehicle was greatest in 3-week-old rats (129 pmol; 64% of the administered dose), decreasing to ~80 pmol (40%) in 5-, 8-, and 10-week-old rats and to 45 pmol (22%) in 36-week-old rats. The results indicate that 2,3,7,8-TCDD is absorbed to a greater degree through skin of very young animals and that a significant decrease in potential for systemic exposure may occur during maturation and again during aging.

1.5. SELECTION OF DOSE SURROGATE

Given the myriad of responses to TCDD and its congeners, the selection of a dose metric for use in quantitative risk assessment is complicated. Dose-response relationships must be considered, and these are discussed in greater detail in Chapter 8.

Over recent years much attention has been paid to intracellular pharmacodynamic responses resulting from tissue exposure to TCDDs, TCDFs, and PCBs. Some of the most prominent pharmacodynamic effects are the Ah-receptor-mediated response by CYP1A1 in the liver, lung, and skin and CYP1A2 in the liver. van Birgelen et al. (1996a) found that for a mixture of PeCDDs, PeCDFs, and PCBs there was a good correlation (less than an order of magnitude) between measured induction of CYP1A1 and CYP1A2 and the predicted values based on the TEQ-dose measure. On the other hand, because of suspected synergistic effects the TEQ-

dose measure does not predict well the hepatic porphyrin accumulation in mice (van Birgelen et al., 1996b).

For TCDD alone there is strong evidence that acute and subchronic toxicities are identical after whole-body pharmacokinetics are considered. Li and Rozman (1995) conclude that body burden alone determines toxicity. They studied various endpoints in rats using doses ranging from 0.2 μg/kg to 115.0 μg/kg. Body weight reduction did not occur until a total dose of between 5 and 10 μg/kg was reached, regardless of either acute or multiple administration. Phophoenolpyruvate carboxykinase (PEPCK) was reduced and elevated serum tryptophane occurred at identical total doses regardless of the mode of administration. EROD activity was induced with the multiple exposure when the total dose approached levels at which a single dose induced EROD activity. After sufficient time for 75% of the quasi-steady-state body burden to be eliminated (about 40 days or two half-lives) the EROD induction was partially reversible. From this study and that of Rozman et al. (1993), the authors conclude that dose × time is equal to toxicity, suggesting that peak concentration alone is not an adequate predictor but that a concentration level must occur for a finite amount of time. Body burden at any time means the cumulative dose minus the portion of dose already eliminated. Using this approach, Li and Rozman (1995) are suggesting that the body burden of the parent compound, in this case TCDD, is the appropriate measure of toxic dose.

In studying mixtures, quantitative measures of congener dose have been made relative to TCDD as TCDD equivalents (TEQs). TEQs are derived by multiplying each compound's concentration by its TEF. The TEF is a compound's potency compared to that of 2,3,7,8-TCDD. Obviously the TEF of 2,3,7,8-TCDD is 1.0. The TEF approach has been used for several of the various putative toxic endpoints. It has a unique advantage in that it can be used to determine a toxic potency of a mixture of these compounds as long as the concentrations of the individual congeners are known. The concentrations can be those in a food mixture or in a target tissue. However, this method assumes no interaction between the different congeners in the mixture. For some endpoints there is evidence that this assumption is reasonable (Schrenk et al., 1994; Nagao et al., 1993; Hurst et al., 1995).

Hardell et al. (1995) determined the concentrations of several dioxins and dibenzofurans in patients with malignant lymphoproliferative diseases and in patients without malignancies. Significantly higher concentrations of 1,2,3,7,8-pentachlorodioxin, 1,2,3,6,7,8-hexachlorodioxin, and 2,3,4,7,8-pentachlorodibenzofuran were found in the patients with malignant lympho-proliferative diseases. Higher (although not significant) mean concentrations of TCDD were also found in these patients. TEQs of PCDD and PCDF were significantly higher in the patients with malignancies. Also, DeVito et al. (1995) report that for several endpoints, including chloracne

and CYP1A1 induction, humans and animals respond at similar body burdens. These examples further illustrate the utility of body burden as a measure of dose.

Likewise Leonards et al. (1995) report a good relationship between bioaccumulation (body burden) of PCBs and relative litter size or kit survival in mink. When a bioaccumulation model was structured on a congener-specific basis and used with the TEFs, the relationship was further improved. Thus, for this noncarcinogenic endpoint the authors report a good dose-effect relationship between body burden and effects on litters.

Rao and Unger (1995) use a competitive binding model for risk assessment purposes. The conclude that Ah receptor affinity is an important physiologic and biochemical parameter for the determination of tissue dose of the various congeners in mixtures. In summary, they appear to base their TEFs on the binding to the Ah receptor.

Kohn (1995) suggests a very specific pharmacodynamic endpoint as an index of toxicity. His model suggests that oxidation of estradiol to DNA-reactive quinones or semiquinones occurs after induction of CYP1A2 by the dioxins. He speculates that this contributes to an increased mutational rate. The TCDD-stimulated production of a peptide ligand to epidermal growth factor receptor activates tyrosine kinase activity. This tyrosine kinase activity in turn may increase the rate of proliferation of susceptible cells.

Because TCDD is slowly eliminated, the body burden does not respond to small fluctuations in dose and is thus a very good indication of average dose. Also, because CYP1A2 induction is responsible for many of the putative toxic responses, and because induction is a function of body burden, it appears that body burden is the optimal dose metric for reversible responses.

Table 1-1. Gastrointestinal absorption of 2,3,7,8-TCDD and related compounds following a single oral exposure by gavage

Chemical	Species (Sex)	Dose (µmol/kg)	Dose (µg/kg)	Vehicle	% Administered Dose Absorbed[a] [Mean (Range)]	Reference
CDDs						
2,3,7,8-TCDD	Sprague-Dawley rat (M)	0.16	50	acetone:corn oil (1:7)	70	Piper et al., 1973
2,3,7,8-TCDD	Sprague-Dawley rat (M/F)	0.003	1.0	acetone:corn oil (1:25)	84 (66-93)	Rose et al., 1976
2,3,7,8-TCDD	Hartley guinea pig (F)	0.005	1.45	acetone:corn oil (1:45)	50	Nolan et al., 1979
2,3,7,8-TCDD	Golden Syrian hamster (M)	2.0	650	olive oil	74	Olson et al., 1980
2,3,7,8-TCDD	Human (M)	0.000003	0.001	corn oil	87	Poiger and Schlatter, 1986
1,2,3,7,8-PeCDD	Sprague-Dawley rat (M/F)	0.03	9.2	corn oil	NR (19-71)	Wacker et al., 1986
OCDD	Fischer 344 rat (M)	0.11 1.1 1.1 11	50 500 500 5000	o-dichlorobenzene:Emulphor (1:1) o-dichlorobenzene:corn oil (1:1) corn oil suspension corn oil suspension	12 15 2 5	Birnbaum and Couture, 1988

Table 1-1. Gastrointestinal absorption of 2,3,7,8-TCDD and related compounds following a single oral exposure by gavage (continued)

Chemical	Species (Sex)	Dose (μmol/kg)	Dose (μg/kg)	Vehicle	% Administered Dose Absorbed[a] [Mean (Range)]	Reference
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.001 0.01 0.1 0.5	0.5 5 50 500	Emulphor:ethanol:water (1:1:3)	78 82 60 47	Diliberto et al., 1990
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	0.1 1.0	30.6 306	Emulphor:ethanol (1:1)	90 90	Birnbaum et al., 1980
2,3,7,8-TCDF	Hartley guinea pig (M)	0.02	6	Emulphor:ethanol:water (1:1:8)	90	Decad et al., 1981a
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	0.1 0.5 1.0	34 170 340	corn oil	~70 ~70 ~70	Brewster and Birnbaum, 1987
PCBs						
3,3′4,4′-T4CB	C57BL mouse (F)	34.5	10,000	corn oil	77	Wehler et al., 1989

[a]Absorption is generally estimated as the difference between the administered dose (100%) and the percent of the dose that was not absorbed. The unabsorbed fraction is estimated as the recovery of parent compound in feces within 48 hours of exposure.
NR = Not reported.

Table 1-2. Percentage of 2,3,7,8-TCDD in the liver of rats 24 hours after oral administration of 0.5 mL of various formulations containing TCDD

Formulation	TCDD Dose (ng)	No. of Animals	Percentage of Dose in the Liver
50% ethanol	14.7	7	36.7±1.2
Aqueous suspension of soil (37%, w/w) that had been in contact with TCDD for:			
10–15 hours	12.7, 22.9	17	24.1±4.8
8 days	21.2, 22.7	10	16.0±2.2
Aqueous suspension of activated carbon (25%, w/w)	14.7	6	≤0.07

w/w = Weight by weight.

Source: Poiger and Schlatter, 1980.

Table 1-3. Dermal absorption of 2,3,7,8-TCDD and related compounds in the rat

Chemical	Dose		% Administered Dose	
	(µmol/kg)	(µg/kg)	Skin Site[a]	Absorbed
2,3,7,8-TCDD	0.00015	0.05	61.73±4.37	38.27±4.37
	0.001	0.32	59.71±1.90	40.29±1.89
	0.01	3.2	72.60±0.41	27.40±0.41
	0.1	32	82.21±2.85	17.78±2.85
	0.5	160	80.92±2.74	19.08±2.74
	1.0	321	82.68±3.69	17.30±3.67
2,3,7,8-TCDF	0.1	31	51.18±11.95	48.84±11.95
	0.5	153	82.14±11.22	17.86±11.22
	1.0	306	88.70±5.17	11.32±5.17
1,2,3,7,8-PeCDF	0.1	34	74.72±3.58	25.27±3.58
	0.5	170	91.67±2.46	8.33±2.46
	1.0	340	84.23±5.44	15.76±5.44
2,3,4,7,8-PeCDF	0.1	34	65.77±4.80	34.19±4.78
	0.5	170	75.50±1.81	24.50±1.80
	1.0	340	81.84±1.67	18.16±1.67

[a]Values are the mean±SD of three to four animals and represent the amount of administered dose of radiolabeled congener remaining at the application site 3 days after dermal exposure.

Source: Brewster et al., 1989.

Table 1-4. Tissue distribution of [^{14}C]-2-3,7,8-TCDD in female Wistar rats[a]

Tissue	Range of 2,3,7,8-TCDD Concentrations (ng/g)
Liver	29.23–30.99
Adipose tissue	3.72–4.14
Adrenal glands	0.89–1.08
Ovaries	0.76–0.96
Thymus	0.60–1.05
Skin	0.64–0.68
Lung	0.32–0.33
Kidney	0.27–0.29
Pancreas	0.21–0.31
Spleen	0.18–0.23
Serum	0.16–0.18
Bone (with marrow)	0.16–0.16
Muscle	0.08–0.12
Brain	0.07–0.09

[a]Distribution was assessed 7 days after a single subcutaneous exposure (3 µg/kg bw).

Source: Abraham et al., 1988.

Table 1-5. 2,3,7,8-Substituted PCDDs and PCDFs in human liver and adipose tissue

	Tissue Concentrations on a Lipid Basis (ppt)			Tissue Concentrations on a Wet Weight Basis (ppt)	
	Fat	Liver	Liver/Fat	Liver[a]	Liver/Fat
TCDD	8.0	16.4	2.05	1.1	0.14
PeCDD	16.4	20.1	1.22	1.4	0.09
HxCDD	94.7	166.8	1.76	11.7	0.12
HpCDD	106.7	1,002.4	9.39	70.2	0.66
OCDD	373.2	4,416.2	11.83	309.1	0.83
TCDF	2.5	5.5	2.20	0.4	0.15
PeCDF	35.2	173.7	4.93	12.2	0.35
HxCDF	41.5	389.5	9.38	27.3	0.66
HpCDF	14.2	218.9	15.42	15.3	1.08
OCDF	4.0	29.7	7.43	2.1	0.52

Values are the mean of 28 people from the Munich area.
[a]Estimated from the % fat in the liver (7.02±5.33%, mean±SD)

Source: Thoma et al., 1990.

Table 1-6. Elimination of 2,3,7,8-TCDD and related compounds from major tissue depots

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
CDDs						
2,3,7,8-TCDD	Wistar rat (F)	0.3 µg/kg, s.c.	liver liver liver adipose	11.5 16.9 13.6 24.5	95% Confidence interval (time period investigated): 10.7-12.3 (10-49 days) 14.0–21.4 (49–91 days) 12.8–14.4 (10–91 days) 22.4–26.8 (14-91 days)	Abraham et al., 1988
2,3,7,8-TCDD	Wistar rat (M)	1.0 µg//kg, i.p.	liver adipose	37.1 53.2	Tissue levels were measured for 20 weeks following exposure	Lakshmanan et al., 1986
2,3,7,8-TCDD	Sprague-Dawley rat (M)	7 or 20 ppb in diet for 42 days	liver	11	85% total dose	Fries and Marrow, 1975
2,3,7,8-TCDD	Sprague-Dawley rat (F)	7 or 20 ppb in diet for 42 days	liver	13	70% of total dose	Fries and Marrow, 1975
2,3,7,8-TCDD	C57BL/6J mice (M) Ah^b/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	8.5 10.3 16.0	Pool size (% of total dose): 36.8 23.6 7.6	Birnbaum, 1986
2,3,7,8-TCDD	C57BL/6J mice (M) Ah^d/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	7.1 7.6 14.9	Pool size (% of total dose): 20.6 31.3 10.2	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J mice (F) Ah^b/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	12.4 13.3 13.2	Pool size (% of total dose): 29.2 30.9 21.4	Birnbaum, 1986

Table 1-6. Elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (continued)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
2,3,7,8-TCDD	DBA/2J mice (F) Ah^d/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	11.9 11.8 12.8	Pool size (% of total dose): 20.2 42.3 26.6	Birnbaum, 1986
2,3,7,8-TCDD	rhesus monkey (F)	25 ppt in diet	adipose	391±88	Mean±SE (n=7)	Bowman et al., 1989
OCDD	Fischer 344 rat (M)	50 µg/kg, i.v.	liver adipose skin	84 38 3 69	Pool size (% of total dose): 72.7 7.1 9.0, 1st component 0.3, 2nd component	Birnbaum and Couture, 1988
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.5 µg/kg, i.v. (0.001 µmol/kg)	liver adipose skin muscle blood	4.5 16.5 57.8 2.5 57.8 1.6 26.7 18.2	1st component 2nd component 1st component 2nd component 1st component 2nd component	Kedderis et al., 1991a
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle blood	0.10 1.25 3.75 0.45 11.09 0.02 0.72 0.02 1.14	Pool size (% of total dose) 29.09 1st component 31.39 2nd component 17.85 6.84 1st component 1.22 2nd component 24.85 1st component 6.73 2nd component 1.31 1st component 0.89 2nd component	Birnbaum et al., 1980

Table 1-6. Elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (continued)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
2,3,7,8-TCDF	C57BL/6J mice (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle	1.9 1.6 0.15 4.0 0.015 1.1	 1st component 2nd component 1st component 2nd component	Decad et al., 1981b
2,3,7,8-TCDF	DBA/2J mice (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose muscle	1.8 7.0 0.02 4.0	 1st component 2nd component	Brewster and Birnbaum, 1988
1,2,3,7,8-PeCDF	Fischer 344 rat (M)	34 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle adrenal blood	1.36 25.72 12.91 1.32 14.53 0.03 6.96 0.14 2.36 0.07 12.42	Pool size (% of total dose): 42.59 1st component 1.27 2nd component 10.19 7.14 1st component 1.49 2nd component 34.81 1st component 7.42 2nd component 0.26 1st component 0.02 2nd component 5.33 1st component 1.29 2nd component	Brewster and Birnbaum, 1988
1,2,3,7,8-PeCDF	Sprague-Dawley rat (F)	4.0 µg/kg, p.o.	liver	3.3	69.8% of total dose	Van den Berg et al., 1989a,b

Table 1-6. Elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (continued)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	34 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle blood 	193 69 0.62 1.23 0.04 0.51 9.84 0.04 1.32 55	Pool size (% of total dose): 67.71 10.53 3.54 1st component 1.37 2nd component 29.40 1st component 2.01 2nd component 0.78 3rd component 3.18 1st component 0.37 2nd component 0.008 3rd component	Brewster and Birnbaum, 1987
2,3,4,7,8-PeCDF	Sprague-Dawley rat (F)	5.6 µg/kg, p.o.	liver	108	78.3% of total dose	Van den Berg et al., 1989b
1,2,3,6,7,8-HxCDF	Sprague-Dawley rat (F)	6.0 µg/kg, p.o.	liver	73	63.4% of total dose	Van den Berg et al., 1989b
PCBs						
3,3′,4,4′-TCB	Sprague-Dawley rat (F)	5 mg/kg/day, p.o., for 21 days	liver adipose	0.8 2.5	21-day exposure produced steady state with 300 ng/g in liver and 8 µg/g in adipose tissue. Elimination was assessed over a 22-day postexposure period.	Clarke et al., 1984
3,3′,4,4′-TCB	ICR mice (M)	8 mg/kg, p.o., every other day for 10 doses	liver adipose serum	2.15 2.60 1.07	Steady-state tissue concentrations: 1.5 µg/g 19.2 µg/g 0.04 µg/mL	Clevenger et al., 1989

i.v. = intravenous; s.c. = subcutaneous; i.p. = intraperitoneal; p.o. = per os.

Table 1-7. Elimination constants and half-lives of various 2,3,7,8-Substituted CDDs and CDFs in hepatic and adipose tissue of marmoset monkeys[a,b]

Congener	Hepatic Tissue			Adipose Tissue		
	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)
2,3,7,8-TCDD[c]	0.0841±0.0109	8.3	6.6–11.1	0.0658±0.0072	10.5	8.7–13.4
1,2,3,7,8-PeCDD[c]	0.0649±0.0101	10.7	8.2–15.4	0.0490±0.0057	14.2	11.5–18.3
1,2,3,4,7,8-HxCDD	0.0702±0.0059	9.9	8.4–11.8	0.0411±0.0083	16.9	12.1–27.9
1,2,3,6,7,8-HxCDD	0.0558±0.0046	12.4	10.7–14.9	0.0373±0.0073	18.6	13.4–30.2
1,2,3,7,8,9-HxCDD	0.0767±0.0078	9.0	7.5–11.3	0.0525±0.0089	13.2	9.9–19.7
1,2,3,4,6,7,8-HpCDD	0.0518±0.0081	13.4	10.2–19.3	0.0372±0.0060	18.6	14.2–27.2
OCDD	0.0089±0.0084	78	27–∞[d]	0.0122±0.0093	101	20–∞[d]
2,3,7,8-TCDF	0.8012±0.0549	<0.87[e]	-<1.00	0.4986±0.0829	1.39	1.05–2.06
1,2,3,7,8-/1,2,3,4,8-PeCDF	0.7476±0.0294	0.93	0.86–1.00	0.4735±0.0408	1.46	1.25–1.76
2,3,4,7,8-PeCDF	0.0786±0.0048	8.8	7.9–10.0	0.0563±0.0059	12.3	10.2–15.5
1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF	0.0307±0.0039	23	18–30	0.0103±0.0074	68	28–∞[d]
1,2,3,6,7,8-HxCDF	0.0486±0.0037	14.3	12.4–16.7	0.0290±0.0091	24	15–62
1,2,3,7,8,9-HxCDF	0.0848±0.0057	8.2	7.2–9.4	not analyzed[f]	NA	NA
2,3,4,6,7,8-HxCDF	0.0373±0.0057	18.6	14.3–26.5	0.0182±0.0082	38	20–327

Table 1-7. Elimination constants and half-lives of various 2,3,7,8-Substituted CDDs and CDFs in hepatic and adipose tissue of marmoset monkeys[a,b] (continued)

Congener	Hepatic Tissue			Adipose Tissue		
	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)
1,2,3,4,6,7,8-HpCDF	0.0186±0.0072	37	21–152	-0.0140±0.0137	∞[d]	54–∞[d]
1,2,3,4,7,8,9-HpCDF	0.0088±0.0127	79	20–∞[d]	0.0011±0.0112	660	30–∞[d]
OCDF	0.0040±0.0096	174	30–∞[d]	-0.0042±0.0148	∞[d]	28–∞[d]

[a]Source: Neubert et al., 1990.
[b]Animals were treated subcutaneously with a single dose of a defined CDD/CDF mixture, and the tissues were analyzed at different times following treatment. Half-lives were calculated from tissue concentrations of the 2,3,7,8-substituted congeners in hepatic and adipose tissue. Values are given as elimination rate constant K_e including estimated SD and half-life including 95% confidence intervals.
[c]Calculated from the time period: >6 weeks after injection.
[d]Calculated half-life is apparently infinite. Data for OCDD and OCDF are unreliable due to delayed absorption.
[e]Not detected in hepatic tissue 6 weeks after treatment; limits of detection used for calculation.
[f]Due to interference.
NA = Not applicable.

Table 1-8. 2,3,7,8-TCDD concentrations in liver and adipose tissue following different doses and calculated concentration ratios (liver/adipose tissue)[a]

Dose (ng/kg)	Number	TCDD Concentration Liver (ng/g)	TCDD Concentration Adipose Tissue (ng/g)	Concentration Ratio: Liver/Adipose Tissue
1	6	0.0031±0.0009	ND	NA
3	6	0.0102±0.0020	0.0139±0.0015	0.74±0.15
10	12	0.0406±0.0121	0.0494±0.0084	0.82±0.20
30	6	0.162±0.032	0.139±0.021	1.16±0.07
100	6	0.699±0.130	0.335±0.065	2.10±0.27
300	6	3.38±0.22	0.819±0.075	4.14±0.31
1000	6	10.7±2.2	2.02±0.17	5.27±0.96
3000	5	27.9±2.4	3.66±0.31	7.65±0.64

[a]Concentrations were measured 7 days after injection.
ND = Not detectable; NA = not applicable.

Source: Abraham et al., 1988.

Table 1-9. Metabolism and excretion of 2,3,7,8-TCDD and related compounds[a]

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
CDDs									
2,3,7,8-TCDD	Sprague-Dawley rat (M)	50 µg/kg, p.o.	NA	NA	NA	4.0	17.4±5.6[c]	NC	Piper et al., 1973
2,3,7,8-TCDD	Sprague-Dawley rat (M)	7 or 72 ppb in diet for 42 days	NA	NA	NA	NA	12	NC	Fries and Marrow, 1975
2,3,7,8-TCDD	Sprague-Dawley rat (F)	7 or 72 ppb in diet for 42 days	NA	NA	NA	NA	15	NC	Fries and Marrow, 1975
2,3,7,8,-TCDD	Sprague-Dawley rat (M, F)	1.0 µg/kg, p.o	NA	NA	NA	9.9	31±6[d]	NC	Rose et al., 1976
2,3,7,8-TCDD	Sprague-Dawley rat (M, F)	0.1 and 1.0 µg/kg/day, 5 days/week for 7 weeks	NA	NA	NA	8.5	23.7	NC	Rose et al., 1976
2,3,7,8-TCDD	Han/Wistar rat (M)	5 µg/kg, i.p.	>90	NA	~70–90	14.1	21.9	NC	Pohjanvirta et al., 1990
2,3,7,8-TCDD	Long-Evans rat (M)	5 µg/kg, i.p.	>90	NA	~20-90	12.0	20.8	NC	Pohjanvirta et al., 1990
2,3,7,8-TCDD	Sprague-Dawley (M)	500 µg/kg, i.p.	100	100	NA	NA	NA	NC	Neal et al., 1982
2,3,7,8-TCDD	C57BL/6J mice (M)	10 µg/kg, i.p.	100	100	85	2.7	11.0±1.2[d]	NC	Gasiewicz et al., 1983a

Table 1-9. Metabolism and excretion of 2,3,7,8-TCDD and related compounds[a] (continued)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,7,8-TCDD	DBA/2J mice (M)	10 µg/kg, i.p.	100	100	82	1.2	24.4±1.0[d]	NC	Gasiewicz et al., 1983a
2,3,7,8-TCDD	B6D2F1J mice (M)	10 µg/kg, i.p.	100	100	86	2.5	12.6±0.8[d]	NC	Gasiewicz et al., 1983a
2,3,7,8-TCDD	C57BL/6J mice Ah^b/Ah^d (M)	500 ng/kg, i.p.	NA	NA	NA	3.1	9.42	NC	Birnbaum, 1986
2,3,7,8-TCDD	C57BL/6J mice Ah^d/Ah^d (M)	500 ng/kg, i.p.	NA	NA	NA	2.1	9.74	NC	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J Ah^b/Ah^d (F)	500 ng/kg, i.p.	NA	NA	NA	5.3	10.40	NC	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J Ah^d/Ah^d (F)	500 ng/kg, i.p.	NA	NA	NA	6.8	11.11	NC	Birnbaum, 1986
2-Iodo-3,7,8-TCDD	C57BL/6J mice (F)	[^{125}I] 0.1 nmol/kg, i.p.	NA	NA	NA	NA	14.2	whole body counting was used to estimate body burden over 30-day period	Leung et al., 1990b
2-Iodo-3,7,8-TCDD	C57BL/6J mice (F)	[^{125}I] 0.1 nmol/kg, i.p., 3 days following pretreatment with 2,3,7,8-TCDD (0.1 µmol/kg, i.p.)	NA	NA	NA	NA	8.0	whole body counting was used to estimate body burden over 30-day period	Leung et al., 1990b
2,3,7,8-TCDD	Hartley guinea pig (M)	0.5 µg/kg, i.p.	NA	NA	NA	15.7	30.2±5.8[d]	NC	Gasiewicz and Neal, 1979
2,3,7,8-TCDD	Hartley guinea pig (M)	0.56 µg/kg, i.p.	100	100	19	11.2	93.7±15.5[d]	NC	Olson, 1986
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{3}H] 650 µg/kg, i.p.	NA	NA	NA	1.4	11.95±1.95[d]	NC	Olson et al., 1980; Neal et al., 1982
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{14}C] 650 µg/kg, i.p.	100	100	55-75	NA	10.82±2.35	NC	Olson et al., 1980; Neal et al., 1982

Table 1-9. Metabolism and excretion of 2,3,7,8-TCDD and related compounds[a] (continued)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{3}H] 650 µg/kg, p.o.	NA	NA	NA	NA	14.96±2.53	NC	Olson et al., 1980; Neal et al., 1982
2,3,7,8-TCDD	human (M)	1.14 ng/kg, p.o.	NA	NA	~50	>3.1	2120[e]	NC	Poiger and Schlatter, 1986; Wendling et al., 1990
2,3,7,8-TCDD	rainbow trout	494 ppt in diet for 13 weeks	NA	~75	NA	NA	105	elimination followed for 13 weeks following exposure	Kleeman et al., 1986b
2,3,7,8-TCDD	yellow perch	494 ppt in diet for 13 weeks	NA	~90	NA	NA	126	elimination followed for 13 weeks following exposure	Kleeman et al., 1986a
1,2,3,7,8-PeCDD	Sprague-Dawley rat (M, F)	8.42–10.06 µg/kg, p.o.	NA	100	NA	12	29.5±2.7	NC	Wacker et al., 1986
OCDD	Fischer 344 rat (M)	50 µg/kg, iv	<33	0	0	>65	~70	whole body $t_{1/2}$ estimated from body burden in liver, skin, and adipose tissue over 56-day period	Birnbaum and Couture, 1988
OCDD	Fischer 344 rat (M)	50 µg/kg/day, p.o., for 10 days	NA	NA	NA	NA	~173	whole body $t_{1/2}$ estimated from body burden in liver, skin, and adipose tissue over 112-day period	Birnbaum and Couture, 1988
BDDs									
2,3,7,8-TBDD	Fischer 344 rat (M)	0.001 µmol/kg, iv	NA	100	80–90	11.1	0.7 2.9 17.8	Pool size (% of dose): 11.63 1st component 2.78 2nd component 1.45 3rd component	Kedderis et al., 1991a
2,3,7,8-TBDD	Fischer 344 rat (M)	0.1 µmol/kg, iv	NA	100	80-90	9.2	0.6 17.8	Pool size (% of dose): 22.47 1st component 2.35 2nd component	Kedderis et al., 1991a

Table 1-9. Metabolism and excretion of 2,3,7,8-TCDD and related compounds[a] (continued)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
CDFs									
2,3,7,8-TCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	100	>96	99	31.4	1.8 0.3	fecal excretion urinary excretion	Birnbaum et al., 1980
2,3,7,8-TCDF	C57BL/6J mice (M)	0.1 µmol/kg, iv	100	NA	80	6.5	2.8 1.8 2.0	urine feces urine and feces	Decad et al., 1981b
2,3,7,8-TCDF	DBA/2J mice (M)	0.1 µmol/kg, iv	100	NA	80	2.8	4.9 5.4 4.0	urine feces urine and feces	Decad et al., 1981b
2,3,7,8-TCDF	Hartley guinea pig (M)	0.02 µmol/kg, iv	>90	NA	<10	1.0	20	animal exhibited body weight loss	Decad et al., 1981a
2,3,7,8-TCDF	Hartley guinea pig (M)	4 µg/kg, p.o.	NA	NA	NA	NA	40	no observable toxicity	Ioannou et al., 1983
2,3,7,8-TCDF	rhesus monkey (M)	0.1 µmol/kg, iv	100	>92	>92	5.4	6.24 10.30 ~8	urine feces urine and feces	Birnbaum et al., 1981
1,2,3,7,8-PeCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	~90	100	NA	12.8	 0.92 3.32 1.26 17.32 1.12 6.30	Pool size (% of dose): <u>feces</u>: 57.79 1st component 6.92 2nd component <u>urine</u>: 2.68 1st component 0.16 2nd component <u>feces and urine</u>: 59.97 1st component 2.51 2nd component	Brewster and Birnbaum, 1988
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	NA	>90	>90	>100	 1.27 63.82	Pool size (% of dose): <u>feces</u> 1.22 1st component 0.57 2nd component	Brewster and Birnbaum, 1987

Table 1-9. Metabolism and excretion of 2,3,7,8-TCDD and related compounds[a] (continued)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,4,7,8-PeCDF	rhesus monkey (M)	0.1 µmol/kg, iv	NA	NA	63–70	~34	38–49	$t_{1/2}$ represents minimum value; all animals lost body weight and exhibited other signs of toxicity	Brewster et al., 1988
CBs									
3,3'4,4'-TCB	CD rat (M, F)	0.6 mg/kg, iv	>90	NA	>90	42	~1.3–1.5	NC	Abdel-Hamid et al., 1981
3,3'4,4'-TCB	rhesus monkey (F)	0.6 mg/kg, iv	97	NA	97	7.2	~8–10	NC	Abdel-Hamid et al., 1981

[a]All studies measure the excretion of radiolabeled parent compound and metabolites following exposure to a single congener labeled with ^{3}H, ^{14}C, or ^{125}I.
[b]Half-life for excretion estimates assume first-order elimination kinetics.
[c](mean±SE).
[d](mean±SD).
[e]n=1.
i.p. = intraperitoneal; i.v. = intravenous; NA = not available; NC = no comment; p.o. = per os.

Table 1-10. Half-life estimates for 2,3,7,8-TCDD and related compound in humans

Chemical	Exposure Incident	Number of Individuals	Sample	Time Period Between First and Last Analysis	Number of Time Points	Half-Life (years)	Reference
CDDs							
2,3,7,8-TCDD	Male volunteer	1	fecal excretion	125 days	28	5.8	Poiger and Schlatter, 1986
2,3,7,8-TCDD	Male volunteer	1	adipose tissue	6 years	5	9.7	Schlatter, 1991
2,3,7,8-TCDD	Ranch Hand Vietnam veterans	36	serum	5 years	2	7.1[a]	Pirkle et al., 1989
2,3,7,8-TCDD	Ranch Hand Vietnam veterans	337	serum	5 years	2	11.3[b]	Wolfe et al., 1994
1,2,3,6,7,8-HxCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	3.5	Gorski et al., 1984
1,2,3,4,6,7,8-HpCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	3.2	Gorski et al., 1984
OCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	5.7	Gorski et al., 1984
CDFs							
2,3,4,7,8-PeCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	4.7 7.2 4.5	Schecter et al., 1990b
1,2,3,4,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	2.9 4.4 4.0	Schecter et al., 1990b
1,2,3,6,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	3.5 4.3 4.9	Schecter et al., 1990b

Table 1-10. Half-life estimates for 2,3,7,8-TCDD and related compound in humans (continued)

Chemical	Exposure Incident	Number of Individuals	Sample	Time Period Between First and Last Analysis	Number of Time Points	Half-Life (years)	Reference
1,2,3,4,6,7,8-HpCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	6.5 4.1 6.8	Schecter et al., 1990a
2,3,4,7,8-PeCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	1.3 2.9 1.7	Ryan and Masuda, 1989
1,2,3,4,7,8-HxCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	2.1 5.1 2.4	Ryan and Masuda, 1989
1,2,3,4,6,7,8-HpCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	1.6 6.1 2.4	Ryan and Masuda, 1989
2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF	Yu-Cheng	3	blood	9 years	5-6	2-3	Ryan and Masuda, 1991
2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF	Yusho	9	blood	7 years	3-5	>5	Ryan and Masuda, 1991
1,2,3,4,6,7,8-HpCDF	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	<1.7	Gorski et al., 1984
OCDF	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	1.8	Gorski et al., 1984
PCBs							
3,3',4,4',5-PeCB	Yu-Cheng	NA	blood	NA	NA	<1	Ryan and Masuda, 1991
3,3',4,4',5,5'-HxCB	Yu-Cheng	NA	blood	NA	NA	10	Ryan and Masuda, 1991

[a]95% confidence interval about the median of 5.8–9.6 years.
[b]95% confidence interval about the median of 10.0-14.1 years.
NA = Not applicable.

Table 1-11. Calculated daily intakes for 2,3,7,8-TCDD

Half-life (yrs)	Fat vol. (L)	Fat conc. (ppt)	Calculated daily intake (PG/KG/Day)
5.8	14.0	6.72	0.44
7.0	14.0	6.72	0.37
5.8	14.0	5.00	0.33
7.0	14.0	5.00	0.27
5.8	7.0	6.72	0.22
7.0	7.0	6.72	0.18
5.8	7.0	5.00	0.16
7.0	7.0	5.00	0.14

Table 1-12. Half-life calculations

Chemical	Food-ppt[a]	Body-ppt[b]	$t_{1/2}$[c] years	$t_{1/2}$[d] years
2378-TCDD	0.23	6.72	7.0	6.0
2378-TCDF	0.84	3.9	1.11	1.3
12378-PeCDD	0.7	21.5	7.35	5.0
23478-PeCDF	1.4	36.8	7.94	6.3
OCDD	19.2	653.0	8.14	50.0

[a]Concentrations in food for TCDD, TCDF, and OCDD were obtained as discussed in Chapter 3; those for PCDD and PCDF were taken from Schlatter (1991).
[b]Concentrations in body (adipose tissue) were all taken from Schecter (1991).
[c]Calculated using Equation 1-19, except for TCDD.
[d]Calculated by Schlatter (1991), except for TCDD.

Table 1-13. Half-life estimates taken from Flesch-Janys et al. (1996)

Compound	N individuals	Median half-life estimate (years)
2,3,7,8-TCDD	48	7.2
1,2,3,7,8-PeCDD	40	15.7
1,2,3,4,7,8-HCDD	41	8.4
1,2,3,6,7,8-HCDD	40	13.1
1,2,3,7,8,9-HCDD	39	4.9
1,2,3,4,6,7,8-HpCDD	26	3.7
OCDD	32	6.7
2,3,4,7,8-PeCDF	5	19.6
1,2,3,4,7,8-HCDF	42	6.2
1,2,3,6,7,8-HCDF	31	6
2,3,4,6,7,8-HCDF	6	5.8
1,2,3,4,6,7,8-HpCDF	22	3
1,2,3,4,7,8,9-HpCDF	6	3.2

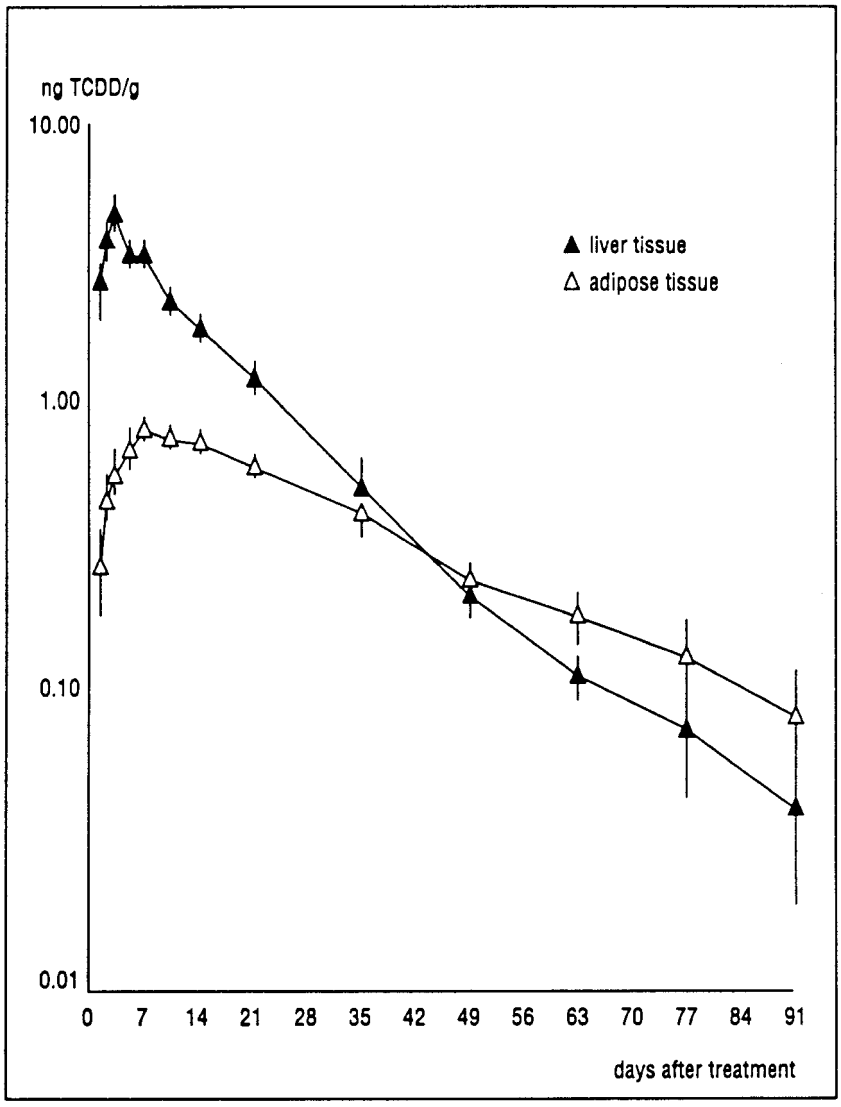

Figure 1-1. Time course of the connection of 14C-TCDD in rat liver and adipose tissue after a single subcutaneous injection of 300 ng TCDD/kg bw to female rats (M±SD).

Source: Abraham et al., 1988.

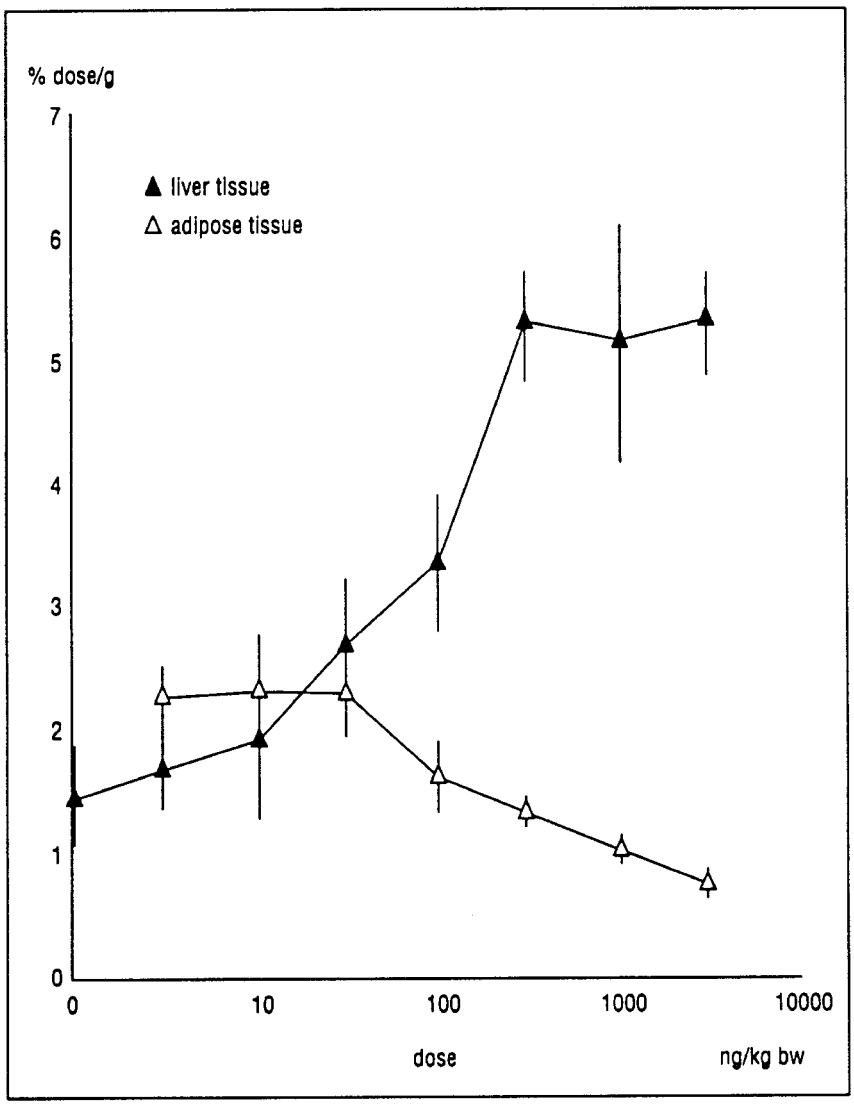

Figure 1-2. Dose dependency of the percentage of the administered dose of 14C-TCDD/g of tissue recovered in liver and adipose tissue after single subcutaneous doses (values from animals treated with 3000 ng TCDD/kg bw were corrected for 84% adsorption). Concentrations were measured 7 days after the injection.

Source: Abraham et al., 1988.

$$D = \left(\frac{ln2}{t_{1/2}} \right) V_F \, C_F \left(\frac{1}{70KG} \right)$$

$$D = \left(\frac{ln2}{5.8years} \right) \left(14L \;\; 1000 \; \frac{ml}{L} \right) \left(6.72 \frac{pg}{ml} \right) \left(\frac{1}{70KG} \right) \left(\frac{1year}{365days} \right)$$

$$D = 0.44 \;\; pg/kg/day$$

Figure 1-3. Sample calculation of daily intake for 2,3,7,8-TCDD.

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